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Extracellular matrix remodelling in rat heart in an unpredictable chronic mild stress model associated with diabetes

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The coexistence of depression and diabetes is a serious challenge in cardiovascular disease. However, extracellular matrix (ECM) under stress model associated or not with diabetes remains unknown. This study aims to investigate the involvement of diabetes and unpredictable chronic mild stress (UCMS) on ECM remodelling. Rats were exposed to UCMS, diabetes or combined treatment. mRNA expression of matrix metalloproteinases (MMP-2 and MMP-9), plasminogen activator (t-PA) and inhibitor (PAI-1) was examined by Q-RT-PCR. ECM was determined by ELISA. MMP-2 and MMP-9 mRNA was lower in diabetes (P <0.05). UCMS increased MMP-2 and MMP-9 compared to control and diabetic group. Compared to the control and diabetes groups, MMP-2 and MMP-9 mRNA was significantly increased in the combined treatment group. UCMS increased MMP-2 expression in the diabetic group (P <0.01). Compared to control, gelatinase activity was higher in diabetes and UCMS (P <0.05). Combined diabetes and UCMS significantly increased gelatinase activity compared to T2D and UCMS groups. Collagen, hydroxyproline and fibronectin content were significantly increased in diabetes and combined groups. UCMS combined with diabetes exacerbated diabetes-induced MMPs and fibronectin deposition. In conclusion, comorbidity between diabetes and UCMS may exacerbate ECM remodelling. These findings will be useful in understanding diabetes-induced cardiovascular disease.

Keywords: Cardiovascular disorder (CVD), Comorbidity, Depression, Gelatinolytic activity, Fibronectin deposition, mRNA expression, matrix metalloproteinases (MMPs), Plasminogen activator (t-PA), Plasminogen inhibitor (PAI-1), Social stress

The balance between synthesis and degrading extracellular matrix (ECM) is important for the structure and function of cardiovascular tissue. Thus, abnormal deposition or degradation of ECM components occurs in cardiovascular disease[1]. Degradation of the ECM is achieved by a tightly regulated family of zinc-dependent endopeptidases called matrix metalloproteinases (MMPs), including MMP-2 and MMP-9, which are secreted as inactive zymogens (pro-MMPs) and require proteolytic activation by plasminogen activator (t-PA) to be functional in tissue[2]. However, t-PA activity is inhibited by Plasminogen inhibitor (PAI-1) and can degrade most components of the ECM and basement membrane, as well as being involved in many other proteolytic processes.

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Diabetes mellitus is a major risk factor for the development of several diseases[3,4] and diabetic patients have a higher risk of developing heart failure[4]. Hyperglycaemia has been shown to increase the expression and activity of MMPs in vascular macrophages and endothelial cells and to promote cardiovascular disease[5].

Epidemiological evidence suggests that people who experience chronic social stress are at increased risk of developing type 2 diabetes (T2D). Furthermore, the presence of diabetes may exacerbate the negative impact of social stress on overall health outcomes[6,7]. Studies exploring the underlying mechanisms of this interaction have identified potential pathways involving dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system[8]. Chronic social stress can activate these stress-responsive systems, leading to elevated levels of stress hormones such as cortisol and catecholamines. Thus, stress-induced behavioural changes may play a critical role in linking stress to

insulin resistance. The influence of stress hormones, including cortisol, epinephrine, growth hormone and glucagon, is widely recognised in causing temporary increases in blood glucose levels, known as acute transient hyperglycaemia[9]. A strong correlation between depressive symptoms and plasma MMP-9 levels has been reported and confirmed in a Swedish population[10]. Furthermore, plasma MMP-9 levels are considered to be one of the strongest markers of major depression and a risk factor for several somatic diseases[11].

However, the expression and activity of MMPs in heart tissue in a stress model combined with type-2 diabetes have not been extensively investigated. Hence, in this study, we tried to elucidate the effects of depression, alone or in conjunction with diabetes, on MMP expression and activity, as well as t-PA and PAI levels in rats.

Materials and Methods

Animals

A total of 30 Wistar rats weighing between 174 and 190 g were used in this study. The animals were maintained under consistent environmental conditions, including a temperature of 23°C, a relative humidity of 70% and a 12 h light/dark cycle. They had *ad libitum* access to a commercial pellet diet and tap water throughout the experiment. After a one-week acclimation period, rats were randomly assigned to one of the four specific protocol groups: Gr. I, control group (CTRL) (n = 10); Gr. II, diabetes group (T2D) (n = 9); Gr. III, UCMS group (UCMS) (n = 10); and Gr. IV, combined diabetes and UCMS group (T2D + UCMS) (n = 9). The animal protocols used in this study were ethically approved by the University Animal Care and Use Committee of the University of Gabes, Tunisia (approval number LNFP/Pro152018) and complied with the guidelines outlined in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 concerning the protection of animals used for scientific purposes.

Diabetes induction

Diabetes was induced in the rats by a single intraperitoneal injection of alloxan (150 mg/kg) purchased from Sigma Chemical Co. (St Louis, MO, USA) dissolved in physiological saline (0.9% NaCl, pH 5.6). The rats were injected after a 12 h fast and then received a 10% glucose solution in their drinking water 24 h after the alloxan injection. Following alloxan administration, all induced rats exhibited hyperglycaemia exceeding 200 mg/dL within 48 h. Blood glucose levels were monitored daily using an electronic glucometer (ACCU-CHEK®, Roche, India) through a small tail puncture. The diabetic rats were divided into two different groups: the T2D group, in which the rats were housed without any additional treatment, and the T2D+UCMS group, which underwent the UCMS protocol.

Chronic unpredictable mild stress protocol

Chronic unpredictable mild stress was applied as previously described[12]. Animals with T2D and T2D+UCMS were housed individually and subjected to different types of stressors in random order for 8 h/day, 5 days/week, for 8 weeks: (i) Wet bedding: Approximately 200 mL of water was added to each standard cage; (ii) swimming in water at room temperature (25°C); (iii) tilting cages to approximately 45° (without bedding); (iv) pairing with another neighboring stressed rat; and (v) no bedding; and light/dark cycle inversion. Rats received one of these stressors per day and the same stressor was not applied for 2 days so that the animals could not predict the occurrence of stimulation. At the end of the treatment, rats were euthanized by an overdose of chloral hydrate and the left ventricle (LV) was removed and stored at −80°C. Blood was collected from the inferior vena cava and serum was obtained by centrifugation (3000 rpm, 15 min, at 4°C) and stored at −80°C until use.

Total RNA extraction and real-time RT-PCR analysis

mRNA expression was assessed according to previous protocol[13]. RNA extraction from the LV was performed using trizol reagent (Invitrogen, Carlsbad, CA), followed by DNase treatment using deoxyribonuclease I, amplification grade (Invitrogen). Subsequently, 5 µg of total RNA was subjected to reverse transcription using superscript II transcriptase (Invitrogen, France) with random hexamers as primers (pdN6; Amersham Biosciences, Piscataway, NJ). Quantitative RT-PCR was conducted on selected gene transcripts using a MyiQ thermal cycler (Bio-Rad Laboratories, Hercules, CA). The resulting cDNA served as the template, and amplification was performed with iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) and the appropriate primer set (Invitrogen, France) (Table 1). Two-step RT-PCR real-time amplifications were carried out with an initial denaturation at 95°C for 3 min,

Table 1 — Sequences of used primers

MMP-2	F: ACGATGGCAAGGTGTGGTGT
	R: CCTTGGTCAGGACAGAAGCC
MMP-9	F: CGAGCTATCCACTCATCAAACAT
	R: GTGTCCTCCGATGTAAGAGAGAA
t-PA	F: TCTTCTGTGGAAGAGGAAGAGG
	R: CTGAACTGGATCCAAGACAACA
PAI-1	F: AGCGACACGGCAACAAGAG
	R: GAAGGGGTGAAGCGAACC
18S rRNA	F: AGTCGGCATCGTTTATGGTC
	R: TGAGGCCATGATTAAGAGGG

followed by 10 s at 95°C and 45 s at 60°C. Duplicate PCR reactions were performed for each sample. Cycle threshold values were determined using Optical System Software (Bio-Rad Laboratories, Hercules, CA). The expression levels obtained from the standard curves were normalized against 18S rRNA.

Protein extraction and total gelatinases activity

Gelatinase activity was determined using the gelatinase assay kit (Fluorometric) (Bio Vision, catalogue number K444-100) according to the manufacturer's instructions and previous protocol[14]. Quick-frozen LV tissue (100 mg) was crushed with a mortar and pestle and homogenised in cell lysis buffer (Cat. No. K444-100-2). The homogenate was centrifuged at 16,000 × g, 4°C for 10 min and the supernatant was stored on ice. Protein concentrations of the supernatants were measured using the BCA kit (Pierce, USA). 50 µg of LV homogenate and 50 µL of purified enzyme were added to a 96-well plate. As a positive control, 2 µL of enzyme positive control was added to 18 µL of gelatinase assay buffer. The gelatinase activity was determined according to the following equation:

$$\text{Sample gelatinases activity (U/mg)} = \frac{(B \times 1000) \times \text{dilution factor} / (A \times C)}{P}$$

where A = slope of the FITC standard curve (ΔRFU/pmol); B = sample gelatinase activity as calculated (RFU/min); C = µL of sample used in the assay; and P = protein concentration in the lysate (mg/mL). 1000 = conversion factor (1000 µL ≡ 1 mL)

Hydroxyproline content assay

Hydroxyproline content in LV homogenate was determined using by the colorimetric assay kit (BioVision, K226-100) as previously described[14]. LV was hydrolyzed with 10 N NaOH at 120°C for 1 h. After neutralisation with hydrochloric acid, the hydrolysates were diluted with distilled water and hydroxyproline was determined by calorimetry at 550 nm with p-dimethylaminobenzaldehyde.

Determination of collagen, fibronectin and laminin content

Enzyme-linked immunosorbent assay (ELISA)

Total collagen, fibronectin and laminin levels was determined in LV homogenates by commercial ELISA kits BioVision (K218-100), Abcam (ab108850) and LifeSpan (LS-F6465) respectively according to the manufacturer's protocol. The data was recorded and calculated using an iMark microplate reader (Bio-Rad).

Collagenase activity

Collagenase activity (MMP -1, -8 and -13) was determined in 100 µg protein of LV homogenates using a collagenase activity colourimetric assay kit (K792, Biovision). LV homogenates were mixed with 98 µL collagenase assay buffer. Then 40 µL of collagenase substrate (FALGPA) and made up to 200 µL with collagenase assay buffer. Absorbance was measured immediately at 345 nm for 5-15 min using collagenase assay buffer as a control. Collagenase activity was determined using the following equation:

$$\text{Sample collagenase activity (U/mL)} = \frac{\left(\frac{\Delta A345}{\Delta T}\text{Sample} - \frac{\Delta A345}{\Delta T}\text{Control}\right) \times (0.2) \times \text{Fd}}{(0.53) \times V}$$

where ΔA345 nm = difference of absorbance at two times points; ΔT = period time between two absorbance reading; 0.2 = total assay volume (mL), Fd = dilution factor; 0.53 = millimolar extinction coefficient of FALGPA; and V = sample volume (mL).

Assay of biochemical serum markers

Serum cholesterol, triglyceride and HDL levels were measured using a turbidimetry analyser (Biosystems SA, Barcelona, Spain) with reference number SN834000228 at the Habib Bourguiba University Medical Centre, Medenine (Tunisia). All reagents were purchased from Biosystems (Spain).

Statistical analysis

Statistical comparisons between treated and control groups were performed using the ANOVA test. Subsequent pairwise comparisons were performed with the HSD Tukey test using Statistica software (Statsoft, France). Results are expressed as mean ± SEM. $P < 0.05$ is taken as a significant probability and n values indicate the number of replicates.

Results

Blood glucose level and lipid profile analyses

Table 2 summarizes the fasting blood glucose levels and lipid profiles obtained from the control and

Table 2 — Biochemical serum parameters from control and treated rats

	CTRL	T2D	UCMS	T2D+UCMS
Fasting blood glucose (mg/dL)	118.22 ±11.04	240.45 ±13.24**	133.47 ±9.5	267.75 ±11.74**$^{\neq}$
Chol Total- mmol/L	1.05±0.05	1.26±0.06*	1.07±0.04	1.31±0.064*
TG mmol/L	2.98±0.87	1.67±1.02	1.99±0.46	2.11±0.73
HDL mmol/L	0.41±0.09	0.44±0.12	0.54±0.17	0.49±0.08

[Data are mean±SEM. *P <0.05, **P <0.01 *vs*. CRTL, $^{\neq}P$ <0.05 *vs*. T2D. CTRL, control; T2D, Alloxan_induced diabetic rats, UCMS, unpredictable chronic mild stress. TC, total cholesterol; TG, triglycerides; HDL, High Density Lipoprotein]

treated groups. The mean fasting blood glucose level in the control rats was 118.22±11.04 mg/dL. However, administration of alloxan resulted in a significant increase in blood glucose levels (P <0.01) from the third day after injection, reaching 240.45±13.24 mg/dL (P <0.01) at the end of the experiments. Treatment with UCMS resulted in a slight, non-significant increase in blood glucose levels (133.47±9.5 mg/dL) compared to the control group. Notably, the combined T2D and UCMS group showed a significant 10% (P <0.05) and 50% (P <0.01) increase in fasting glucose levels compared to the T2D and UCMS groups, respectively.

When analyzing the lipid profiles of all groups, it was observed that the serum total cholesterol level was significantly higher (P <0.05) in the T2D rats (1.26±0.06 mmol/L) and in the combined diabetes and UCMS group (1.31±0.064 mmol/L) compared to the control group (1.05±0.05 mmol/L). However, no significant differences in triglyceride and HDL levels were observed between the T2D, UCMS and combined treatment groups (Table 2).

MMP2 and MMP-9 mRNA expression response

Figure 1 shows the mRNA expression levels of MMP-2 and MMP-9 in the four experimental groups. Compared to the control group (CTRL), T2D showed a significant decrease in MMP-2 and MMP-9 mRNA expression by 29% and 20%, respectively (P <0.05). Conversely, UCMS treatment resulted in an increase in MMP-2 and MMP-9 expression of 35% (P <0.05) (Fig. 1A) and 135% (P <0.01) (Fig. 1B), respectively, compared to the CTRL and T2D groups. Notably, the combined T2D and UCMS group showed a significant increase in MMP-2 and MMP-9 mRNA expression compared to both the control and diabetic groups (P <0.01). Furthermore, the combined diabetes and UCMS treatment specifically increased MMP-2 mRNA expression (Fig. 1A) (P <0.01) compared to the UCMS group, whereas no significant changes in MMP-9 mRNA expression were observed (Fig. 1B).

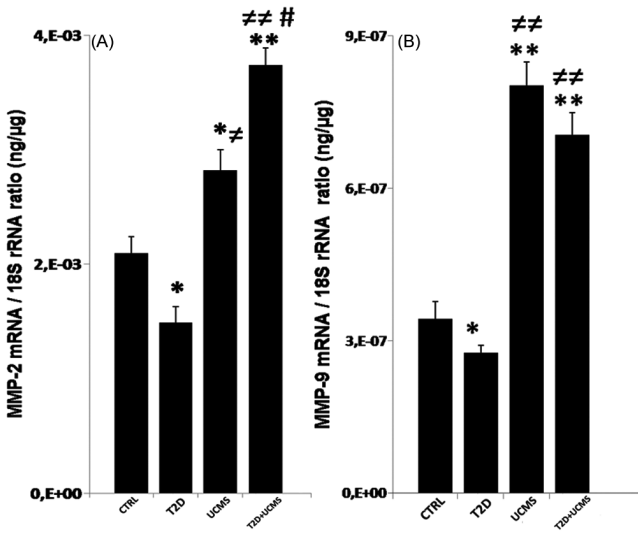

Fig. 1 — Quantitative real-time RT-PCR of (A) MMP-2; and (B) MMP-9 mRNA in the LV from control and treated rats. [Values are presented as ratio of mRNA to 18S rRNA. Data are mean±SEM; n=7 in each group. *P <0.05, **P <0.01 *vs*. CRTL, $^{\neq}P$ <0.05 $^{\neq\neq}P$ <0.01; *vs*. T2D; $^{\#}P$ <0.05 *vs*. UCMS. CTRL, control; T2D, Alloxan_induced diabetic rats, UCMS, unpredictable chronic mild stress exposed rats; T2D+UCMS, combined treated rats; LV, left ventricle]

t-PA and PAI-1 mRNA expression response

Figure 2 shows the response of t-PA and PAI-1 mRNA expression in the four experimental groups. Compared to the control group (CTRL), T2D showed a significant increase in t-PA mRNA expression of 114% *(P* <0.01), whereas UCMS treatment resulted in a decrease of 21% *(P* <0.05) (Fig. 2A). The combined UCMS and T2D group showed significantly higher t-PA mRNA expression compared to both the control and UCMS groups (P <0.05), whereas it remained similar to the T2D group. Furthermore, the combination of T2D and UCMS increased t-PA mRNA expression by 67% (P <0.05) compared to the UCMS group, but showed no significant difference compared to the T2D group (Fig. 2A).

Figure 2B shows the response of PAI-1 mRNA expression in the treated and control animals. T2D resulted in a decrease of 30% (P <0.01), whereas UCMS resulted in an increase of 213% (P <0.01) in PAI-1 mRNA expression compared to the control group. Notably, the combined T2D and UCMS group showed a significant increase in PAI-1 expression of 115% (P <0.01) compared to the control group and 207% (P <0.05) compared to the T2D group. However, the combined T2D and UCMS treatment resulted in a 31% (P <0.05) decrease in PAI-1 mRNA expression.

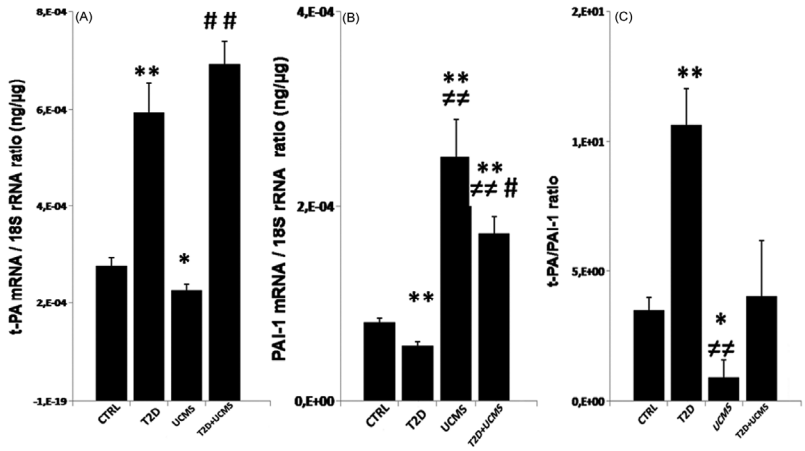

Fig. 2 — Quantitative real-time RT-PCR of (A) t-PA; (B) PAI-1; and (C) mRNA and t-PA/PAI-1 ratio in the LV from control and treated rats. [Values are presented as ratio of mRNA to 18S rRNA. Data are mean±SEM; n=7 in each group. *P <0.05, **P <0.01 *vs*. CRTL, $^{\neq\neq}P$ <0.01 *vs*. T2D; $^{\#}P$ <0.05, $^{\neq\neq}P$ <0.01 *vs*. UCMS. CTRL, control; T2D, Alloxan_induced diabetic rats, UCMS, unpredictable chronic mild stress exposed rats; T2D+UCMS, combined treated rats; LV, left ventricle]

Furthermore, the t-PA/PAI-1 ratio (Fig. 2C) was significantly reduced by 3-fold in the T2D group compared to the control group, but remained statistically unchanged after the combined T2D and UCMS treatment compared to the control group. UCMS alone resulted in a 3.5-fold reduction in the t-PA/PAI-1 ratio compared to the control group.

Total gelatinases and collagenases activities

Gelatinase activities (MMP-2 and MMP-9), and collagenase activities (MMP-1, MMP-8 and MMP-13) were assessed in heart tissue homogenates. The gelatinase activity in heart tissue showed a significant increase of 26% (P <0.01) and 16% (P <0.05) in the diabetes and UCMS groups, respectively, compared to the control group. Notably, the combined diabetes and UCMS treatment group showed a significant increase in gelatinase activity of 63, 29 and 41% (P <0.01) compared to the control, T2D and UCMS groups, respectively (Fig. 3A).

When collagenase activity was examined in the left ventricle (Fig. 3B), T2D and the combined treated group showed a significant increase of approximately 30% (P <0.05) compared to the control group, whereas the UCMS group showed no significant difference from the control group in terms of collagenase activity.

ECM compounds levels

Levels of total collagen, hydroxyproline, fibronectin and laminin were assessed in LV homogenates. Total collagen levels showed a significant increase of 70% in the T2D group and 87% in the T2D+UCMS group compared to the control group (P <0.01 for each). However, exposure to UCMS alone did not induce a significant change in total collagen levels (Fig. 4A).

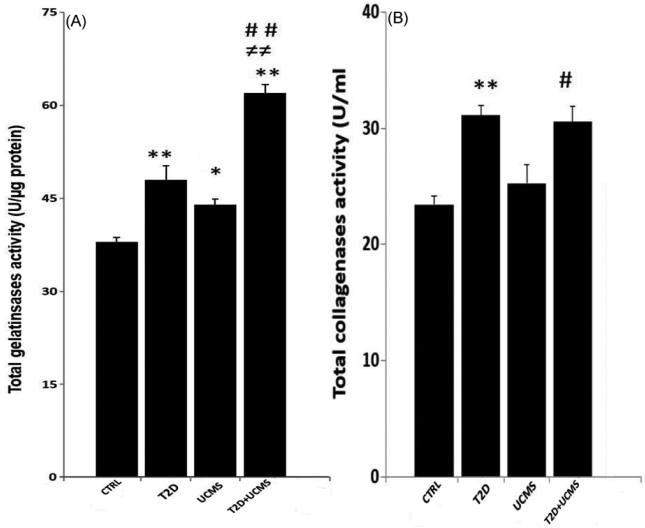

Fig. 3 — (A) Total gelatinases; and (B) collagenases activities in LV homogenates from control and treated rats. [Values are presented as ratio of mRNA to 18S rRNA. Data are mean±SEM; n=7 in each group. *P <0.05, **P <0.01 *vs*. CRTL, $^{\neq\neq}P$ <0.01 *vs*. T2D; $^{\neq}$ $^{\neq}P$ <0.01 *vs*. UCMS. CTRL, control; T2D, Alloxan_induced diabetic rats, UCMS, unpredictable chronic mild stress exposed rats; T2D+UCMS, combined treated rats; LV, left ventricle]

Similar to the collagen results, both T2D and the combined treatment significantly increased LV hydroxyproline content by approximately 2-fold (P <0.01 for each). Conversely, exposure to UCMS alone did not result in any significant changes in hydroxyproline content (Fig. 4B).

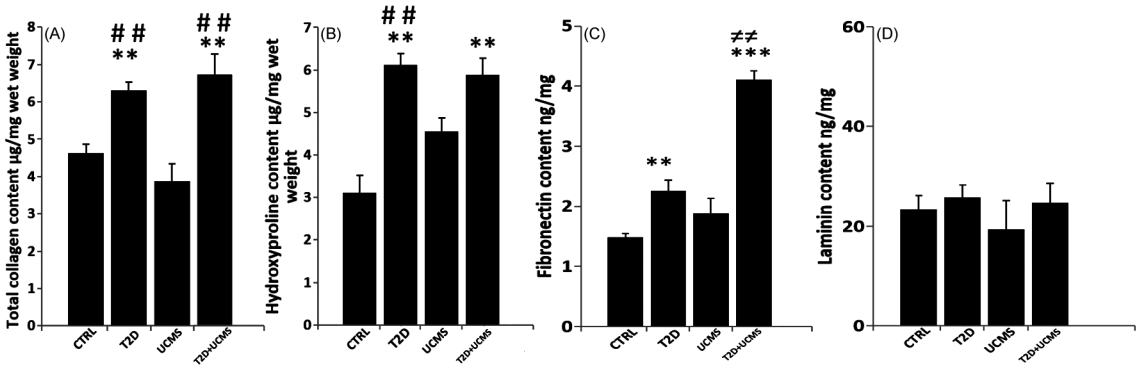

Fig. 4 — (A) Total collagen; (B) Hydroxyproline; (C) Fibronectin; and (D) laminin from control and treated rats. [Data are mean±SEM. ***P* <0.01, ***P* <0.001 *vs*. CRTL, $^{\neq\neq}P$ <0.01 *vs*. T2D; $^{\neq\neq}P$ <0.01 *vs*. UCMS. CTRL, control; T2D, Alloxan_induced diabetic rats, UCMS, unpredictable chronic mild stress exposed rats; T2D+UCMS, combined treated rats]

Figure 4C shows the fibronectin content in the LV of control and treated rats. It was observed that diabetes significantly increased fibronectin content by 50% (*P* <0.01). Furthermore, combined UCMS and diabetes treatment further improved fibronectin levels by approximately 3-fold (*P* <0.001) and 2-fold (*P* <0.01) compared to the control and T2D groups, respectively.

In contrast, LV laminin content remained unchanged in all treated groups compared to the control group (Fig. 4D).

Discussion

In this study, we investigated the involvement of diabetes and social stress, separately or in combination, on ECM remodelling in the rat heart.

We are the first to investigate the response of ECM synthesis and degradation mechanisms in the heart in combined diabetes and stress in an animal model. In this study, we have shown that T2D significantly decreases the expression of MMP-2 and MMP-9 mRNA compared to the control group. MMPs have been widely implicated in the degradation of ECM in tissues. MMP-2 and MMP-9 are zinc-dependent endopeptidases that are secreted as inactive zymogens (pro-MMPs) and require proteolytic activation by t-PA to be functional in tissues. These results confirm previous studies showing that fibrosis in diabetic cardiomyopathy is associated with a decrease in MMP-2 expression[15]. Furthermore, an ECM imbalance has been reported in the regulation of wound healing in diabetic animal models or in diabetic humans, in association with disturbed redox signalling and increased oxidative stress, which contribute to the development of diabetic complications[16]. The observed effect of diabetes on MMP-2 and MMP-9 expression in the heart may explain in part several previous observations that the balance between synthesis and degradation of the ECM maintains the homeostasis of cardiovascular tissue and that abnormal deposition or degradation of ECM compounds occurs in cardiovascular disease[17].

Our results corroborate previous studies showing an increase in serum total cholesterol levels in diabetes[18]. Therefore, it has been established that insulin deficiency or insulin resistance is associated with symptoms of hypercholesterolemia and hypertriglyceridemia[19]. Previous studies in humans have shown that high serum lipid levels in diabetics are caused by increased mobilisation of fatty acids from adipose tissue[20]. Therefore, it has been reported that insulin resistance may be responsible for hyperlipidaemia because insulin has an inhibitory effect on 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-COA reductase), a key enzyme in the biosynthesis of cholesterol[21].

Total gelatinase activity was increased in heart homogenates from diabetic rats. There are few studies in the literature on the effect of diabetes on MMP activity in the cardiovascular system. However, our findings corroborate previous research indicating that diabetes induces increased gingival collagenase activity both in vivo and in vitro and that higher MMP9 activity was identified in wound fluid from biopsy skin samples of human diabetic foot ulcers[22].

Increased gelatinase activity correlated with our t-PA and PAI-1 mRNA expression results. Thus, we found that diabetes increased t-PA expression and decreased PAI-1 expression. Furthermore, our results showed that the t-PA/PAI-1 ratio was significantly increased in diabetic rats. It's well known that MMPs, including MMP-2 and MMP-9, are secreted as inactive zymogens (pro-MMPs) and require

proteolytic activation by t-PA to be functional in tissues[23].

Diabetes and UCMS, separately or in combination, increased the levels of ECM compounds in the heart. Hydroxyline is implicated in collagen synthesis processes by converting procollagen to collagen[24]. Increased collagen levels in diabetic rats confirm previous studies reporting fibrosis in the lungs of 61 diabetic and 50 non-diabetic patients[25]. Furthermore, previous studies reported decreased fibroblast adhesion, decreased response to growth factors and cytokines, and decreased production of collagens and fibronectin in wound healing[26], and diabetes has been described to accelerate changes in collagen metabolism[27].

Fibronectin and laminin are mostly present in the basal lamina of cardiovascular tissue which provide to tissues cell-matrix adhesion. Several pathological processes as cancers and cardiovascular disorders were associated with changes in these two glycoproteins levels. Thus, higher content of fibronectin into sub-endothelial space at atheromatous plaque in aorta was reported to be linked to the activation of pro-inflammatory signaling pathways[28].

We were interested in understanding the effect of the stress model on the expression and activity of MMPs and the expression of t-PA and PAI-1 in cardiac tissue. The World Health Organization ranks depression as the fourth leading cause of disability worldwide and it is considered a very serious medical condition with a lifetime prevalence ranging from about 11% in low-income countries to 15% in high-income countries[29]. In addition, neuropsychiatric manifestations are associated with higher mortality rates worldwide and approximately 450 million people suffer from mental or behavioural disorders[30]. The UCMS model is an established rodent model used to induce depressive-like behaviours by chronically exposing the animal to various random environmental and social stressors[31].

We are the first to assess the modulation of MMPs expression and activity in the heart under stress conditions. Our results indicate that UCMS significantly increases the mRNA expression and activity of MMP-2 and MMP-9. In contrast, UCMS decreased t-PA mRNA expression and increased PAI-1 mRNA expression. These findings are consistent with a previous study showing that PAI-1 mRNA was decreased after chemical sympatectomy in rats and that the sympathetic nervous system stimulates PAI-1 expression[13]. Previous investigations suggest that stress induces extensive ECM remodelling in the hippocampus, which is associated with over-expression of MMPs, and that both expression and activity of MMP-9 occur during the late phase of long-term potentiation (LLTP) at CA3-CA1 synapses in the hippocampus[32].

Paradoxically to the T2D group, we found that a reduced t-PA/PAI-1 ratio in the UCMS group was associated with increased gelatinase activity. This paradox can be explained by the presence of other MMPs activators such as endoproteinase and chemical activation by reactive oxygen species or non-physiological agents. Therefore, lower levels of antioxidants and higher levels of MDA imply the high degree of oxidative stress in unipolar depression[33].

In this study, we were also interested in understanding to what combination of diabetes with UCMS can modulate MMPs expression and activity and t-PA and PAI-1 expression. We chose to investigate the effect of comorbidity between diabetes and UCMS firstly because there are no previous studies reporting the response of MMPs and their activation mechanism under this condition and secondly because in condition where depression and diabetes occur simultaneously represent a serious challenge in clinical diagnosis and treatment because depression and diabetes may be mutually causal and depression is an important risk factor for the aggravation of diabetes[34]. Human studies confirmed a correlation between depression, stress and diabetes. Thus, the prevalence of depression increases in prediabetic and undiagnosed diabetic patients, and significantly increases in previously diagnosed diabetic patients compared to individuals with normal glucose metabolism[35].

Our results show that fasting blood glucose was significantly higher in the combined model of diabetes and UCMS than in control and diabetic rats. Previous studies have stated that anxiety can lead to the release of sympathetic hormones, which can increase both cortisol and glucose levels, decrease insulin release, or affect insulin hormone sensitivity and resistance[36]. Furthermore, there is a strong correlation between depression, anxiety and stress with adherence to lifestyle changes and glycosylated haemoglobin levels followed by depression and stress assessment in African American adults with type 2 diabetes[36,37].

In our model, we found that comorbidity between diabetes and UCMS significantly exacerbates MMP-2 mRNA expression induced by single exposure to UCMS. However, MMP-9 expression does not change in diabetic animals exposed or not exposed to UCMS. In addition, higher levels of t-PA and PAI-1 expression were observed in our combined rat model. Gelatinolytic activity in heart homogenates was higher in diabetic rats co-exposed to UCMS. A previous study reported that the mRNA expression of MMP-9 was significantly higher in patients with depression than in a control group[38]. In addition, the prevalence of depression and elevated depressive symptoms has been reported to be higher in patients with coronary heart disease than in the general population, and depressive symptoms are also associated with an increased risk of cardiac disease. The interaction between diabetes and depression may be related to an inflammatory response, as diabetes is widely recognized as a state of chronic systemic inflammation[39].

The increased expression and activity of MMPs in our model of comorbidity between diabetes and UCMS may in part be related to the activation of inflammatory pathways, contributing to insulin resistance and the development of several cardiovascular disorders. In this way, several biological mechanisms have been proposed to explain the association between depression and anxiety and increased risk of cardiovascular disorders, including alterations in the autonomic nervous system and elevated pro-inflammatory cytokines[40]. We observed increased PAI-1 mRNA expression after UCMS exposure in normal and diabetic rats. This corroborates previous findings[41] showing increased fibrinogen and PAI-1 levels in depressed subjects.

Clinical significance

It is widely recognized within the scientific community that depression serves as an autonomous risk factor for the emergence of cardiovascular disorders. Nevertheless, the intricate interplay between diabetes and depression has not been subject to exhaustive investigation. Within the confines of our study, we have presented compelling evidence elucidating the compromised expression of ECM, activities of MMPs, and the expression of t-PA and PAI-1 in the context of diabetes associated with UCMS. These perturbations play a significant role in the development of mechanical dysfunctions, ultimately leading to various cardiac pathologies. Consequently, our findings assume paramount significance in enhancing our understanding of psychiatric disorders among individuals afflicted with diabetes, thereby facilitating improved preventive strategies.

Conclusion

In the past few decades, substantial studies have provided compelling evidence supporting the premise that depression constitutes an autonomous risk factor for the development of cardiovascular disorders. However, no previous investigations have specifically examined the regulatory mechanisms underlying tissue protease activity and ECM compound levels in the heart within stress models, whether in conjunction with diabetes or independently. In this study, firstly, we observed a marked exacerbation of MMP-2 mRNA expression following exposure to UCMS in rats with concurrent diabetes, thus highlighting the potentiated effect of comorbidity on the UCMS-induced upregulation of MMP-2. Secondly, we noted heightened gelatinolytic activity in cardiac homogenates of diabetic rats simultaneously exposed to UCMS, indicating an augmented proteolytic milieu. Lastly, we identified impaired expression levels of ECM components, perturbed protease activity, and dysregulated expression of t-PA and PAI-1 in the context of diabetes-associated UCMS, all of which play substantial roles in extracellular remodeling within the cardiac tissue micro-environment. These findings hold considerable implications for enhancing our understanding of the heightened risk of depression-induced cardiovascular disorders among diabetic patients. Furthermore, further investigations targeting ECM remodeling and exploring the expression and activity profiles of additional MMPs are warranted to gain deeper insights into the complex interplay between psychiatric disorders, normophysiological conditions, and pathological states such as diabetes.

Conflict of interest

Authors declare no competing interests.

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