RAS-Related C3 Botulinum Toxin Substrate 1 Inhibition Attenuates Platelet Chemokine Activation in Diabetes Mellitus

Keywords: Rac1; Platelet; CXCL4, CCL5; Diabetes Mellitus

ABSTRACT

Diabetes mellitus (DM) is a prevalent cause of platelet (PLT) activation. Inflammation-induced dysregulated PLT function adds to chronic problems. Ras-related C3 botulinum toxin substrate 1 (Rac1), a 21 kDa G-protein, has been shown to modulate many PLT activities; we hypothesized that Rac1 may influence the PLT release of CXCL4 and CCL5, contributing to macrovascular and microvascular problems in DM. The study included Swiss albino male mice pretreated with the Rac1 inhibitor NSC23766 and streptozotocin (STZ) to induce diabetes. A sample of 150 diabetic patients and 50 healthy controls was also analyzed. Statistical analyses were performed using Mann-Whitney tests One hundred fifty confirmed diabetic patients which they visit Layla Qasim health center for diabetes and 50 healthy individuals were included in this study. The serum CXCL4 and CCL5 in diabetic patients and healthy volunteer were measured. Swiss albino male mice were given a pretreatment of 5 mg/kg of the Rac1 inhibitor NSC23766 and then injected with streptozotocin at a dosage of 45 mg/kg body weight, twice daily for five days. Rac1 activity in the PLT was measured using pulldown assay and western blot method. Blood chemokine concentrations were also assessed using ELISA, and histological scores for the kidney, liver, pancreas, and lung were evaluated. CXCL4 and CCL5 levels were markedly elevated in DM patients relative to healthy persons. Our findings indicated that streptozotocin developed diabetes mellitus in mice. GTP-Rac1 was induced in diabetic mice and pretreatment with NSC23766 was significantly lower compared to vehicle group. Furthermore, compared to the sham group, diabetic mice had significantly greater levels of CXCL4 and CCL5 ($P < 0.05$). CXCL4 levels were reduced by 80% following Rac1 inhibition ($P < 0.05$), while CCL5 levels decreased by 55.5% (P

< 0.05). The current research indicates that Rac1 plays a pivotal role in releasing PLT chemokines due to diabetes-induced inflammation in several organs, and inhibiting Rac1 may represent a novel therapeutic approach to managing inflammation in diabetic individuals.

1. Introduction

Diabetes mellitus (DM) is a metabolic illness marked by elevated blood glucose (BG) levels, which may significantly impact several organs in the body (1). The global prevalence of diabetes is on the rise, with an estimated 439 million individuals projected to have the condition by 2030 (2). Chronic hyperglycemia, a characteristic of diabetes, exacerbates vascular complications and elevates morbidity and death rates (1). One of the main risk factors for diabetes is cardiovascular disease (CVD), and it has been shown that hyperglycemia increases the generation of reactive oxygen species (ROS), especially in the blood vessel lining (3).

Diabetic patients also exhibit a hypercoagulable state, with increased platelet (PLT) adhesion and aggregation. Platelet-derived microparticles (PMPs) are formed during PLT activation and have been implicated in various conditions (4). In diabetes, high levels of cell adhesion molecules are observed, and macrophages and monocytes play an essential role in the progression of microvascular complications. Chemokines such as CXCL4 and CCL5, which are small cytokines involved in leukocyte activation and inflammation, are stored in PLT secretory α-granules and attract neutrophils and monocytes (5).

Type 2 diabetes (T2DM) is often accompanied by dyslipidemia, characterized by abnormal lipid profiles with elevated low-density lipoprotein (LDL) and triglyceride levels, and decreased high-density lipoprotein (HDL) levels. Dyslipidemia is a significant risk factor for coronary artery disease (CAD) and is likely caused by insulin's effects on liver apo protein formation, lipoprotein lipase control, and peripheral effects on muscle and adipose tissue. Regulation of lipid profiles is crucial in managing diabetes and reducing complications and mortality rates (6).

The liver is also affected by diabetes. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamyltransferase (GGT) are specific markers of hepatic injury, and elevated serum levels of these enzymes are frequently observed in diabetes. T2DM is associated with liver diseases such as cirrhosis, nonalcoholic fatty liver disease

(NAFLD), nonalcoholic steatohepatitis (NASH), and ultimately hepatocellular carcinoma (7).

Rac1, a small G protein, plays an essential role in intracellular signaling pathways leading to NADPH oxidase activation, which generates ROS. Rac1 activation is essential for the assembly of NADPH oxidase subunits and subsequent ROS production. However, the activation of Rac1 and its impact on PLT chemokines in diabetes are not yet fully understood. Previous studies have shown the involvement of Rac1 in PLT chemokine secretion and activation in other conditions such as septic lung injury and pancreatitis (8). While the role of PLTs in diabetes is well-documented, the specific contribution of Rac1 to PLT chemokine secretion remains unexplored, representing a significant gap in our understanding and for the first time in the current study, the role of Rac1 in PLT chemokine secretion in diabetes is determined.

Objectives

The current study aimed to evaluate PLT chemokines in diabetic patients and investigate the role of Rac1 in DM through the activation of PLT chemokines CXCL4 and CCL5. Furthermore, the current study aimed to evaluate the impact of Rac1 on morphological changes in DM and elucidate the underlying mechanisms associated with diabetes and its complications.

2. Material and Method

2.1. Animals

Swiss albino male mice, aged 8 to 9 weeks, 20 to 25 g, were used in all experiments. The Regional Animal Experimentation Ethical Committee has approved the pharmacy department at Hawler Medical University in Iraq, which complies with laws pertaining to animal welfare standards. Patients having an HbA1c > 6.5% and a verified diagnosis of R2DM were included. The mice were maintained in a pathogen-free setting with a 12-hr light and 12-hr dark cycle. Food and water were supplied twice a day. A particular nipple employed for ad libitum access was supplied with clean and fresh drinking water. Following a seven-day acclimatization phase, the trials were held. The animals were

divided into three groups: sham (saline alone injection), vehicle (streptotocin injection), and treatment (NSC+STZ) which included pretreatment with NSC23366 and streptozotocin. A maximum of five mice per group were housed in cages with environment enrichment. There were five mice in each group.

2.2.Materials

The chemical used to induce diabetes mellitus was streptozotocin (STZ; Glentham Life Science, Ltd., U.K.) (9). To make NaOH, the buffer used to prepare streptozotocin, 10.7 g of sodium citrate was dissolved in 200 ml of distilled water. Then, 9.6 g of citric acid was added, and distilled water was added to get up to 1,000 ml. The solution's pH was brought down to 4.5 by adding NaOH to the solvent. Chem Cruz, Santa Cruz Biotechnology, California; NSC23766 (N6- [2- [[4- (Diethylamino) - 1- methylbutyl] amino] - 6- methyl- 4- pyrimidinyl] - 2 methyl- 4, 6- quinolinediamine trihydrochloride). The BG meter Accu-Chek Active.

2.3.Animal experiments

An intraperitoneal injection of 5 mg/kg of Rac1 inhibitor (NSC23766) was given to the animals. This dosage of NSC23766 was selected according to previous studies (10). After half an hr, the experimental mice were given numerous intraperitoneal injections of STZ (45 mg/kg) to induce diabetes mellitus. Mice received STZ for five consecutive days after it had been dissolved in a buffer containing 0.01 M sodium citrate (pH = 4.5). This STZ dosage was chosen based on earlier research (11). Subsequent to the injection, the animals were administered a glucose solution (5 percent w/v) for nighttime consumption to mitigate the hypoglycemia induced by STZ. Only an equal dosage of the vehicle (citrate buffer) was administered to sham mice. Following dosing, mice treated with STZ and NSC+STZ were kept in their usual environments for five days. Mice acquired diabetes after these times, as shown by fasting BG levels of around 11.1 mmol/l. After a 12-hr fast, blood samples were collected from the tail vein of mice administered STZ and NSC+STZ to assess blood glucose levels. In diabetic mice, fasting BG levels of more than 11.1 mmol/l were considered to be indicative of diabetes (11). They were thus

chosen for more research. 75 mg of ketamine hydrochloride (HoffmanLa Roche, Basel, Switzerland) and 25 mg of xylazine (Janssen Pharmaceutica, Beerse, Belgium) kg-1 were administered intravenously to induce sedation. Vena Cava's blood was extracted and the animals were harvested. The serum was let to coagulate at ambient temperature for ten to twenty minutes. Prior to being centrifuged at 2000–3000 RPM for 20 min. After being separated from the supernatant, the serum was stored at 80 °C in preparation for a subsequent ELISA test. The kidney, liver, lung, and pancreas were kept in formaldehyde for histology.

2.4.Biochemical determination

The BG levels of every experimental animal were checked before the process began. Regular fasting BG checks were performed until diabetes was diagnosed. Mice were considered diabetic if their fasting BG level was 11.11 mmol/l or above. All experimental animals had their tail veins used to draw blood (2–3 µl). The BG levels were tracked using an Accu-Chek active BG meter.

2.5.ELISA

Serum levels of CXCL4 and CCL5 were effectively measured in all groups of diabetes patients, healthy controls, and mice. The animal samples assessed by ELISA using the (Mouse Platelet Factor 4 ELISA Kit, BT LAB Cat. No E0686Mo) and (Bender MedSystems, Vienna, Austria kit.) Diabetic patients and control Serum CXCL4 and CCL5 levels were measured by enzyme-linked immunosorbent assay (ELISA) using the (Human Plt Factor 4 ELISA Kit, BT LAB Cat. No E5885Hu) and (Human regulated activation in normal T-cell expressed and secreted, RANTES, SUNLOG Cat. No SL1526Hu). Each microplate had a standard curve generated by diluting a known concentration standard per the manufacturer's guidelines, with absorbance measured at 450 nm. The chemokine concentration of each sample was determined by computing the mean absorbance for the wells by a logistic curve-fitting method. All absorbance values were located inside the linear segment of the standard curve. The data was represented in both ng/ml and pg/ml, respectively.

2.6.Histopathology

Kidney, liver, lung, and pancreatic tissues were maintained overnight in a 10% formaldehyde phosphate buffer before dehydration and paraffin embedding. Four-micrometer sections were stained with hematoxylin and eosin. A modified scoring system was used to evaluate renal injury in a blinded manner, including mixed inflammatory cells, necrosis (irreversible damage), apoptosis, fibrosis, vascular congestion, edema, and degeneration (irreversible damage). Infiltration is evaluated on a scale from 0 to 4, with zero denoting absence and four representing substantial infiltration (12). Histopathological grading used a modified approach that assessed necrosis, inflammation, and fibrosis on a scale from 0 to 4, where 0 signifies no harm and 4 denotes significant injury. The mean value was determined after evaluating five random sites within each tissue sample. The aggregate of all six criteria establishes the histopathological score.

2.7.Statistics

The data was presented as mean values accompanied with standard error (SEM). Non-parametric tests were selected due to the non-normal distribution of the data. Statistical analyses were conducted using nonparametric testing (Mann-Whitney). N is the total count of mice in each group, and a P-value of less than 0.05 was deemed significant. Statistical analysis was conducted using SPSS (IBM Corp., Armonk, N.Y., USA).

2.8. **Ethical Considerations**

Ethical permission for the human research was obtained from the Institutional Review Board of Layla Qasim Health Center, and all individuals furnished signed informed consent.

3. Results

In 150 DM patients, we assessed clinical and laboratory data. 46% (69) of the total patients were male, and 54% (81) of the total subjects were female (Table 1). The age range and mean ages of the diabetic patients were 8–76 years and 52.185±2.275 years, respectively. The BG profile results revealed that everyone was hyperglycemic. Table 1

displays the group's overall HbA1c percentage and fasting blood sugar (FBS) levels. The control group (CG) are healthy without diabetes with age range and mean age 9-78 years and 50.560±2.773 respectively.

Table 1: Basic characteristics of participants

Variables	Non-Diabetes (n=50)	Diabetes (n=150)	P-Value
Sex			
Male	22	69	
Female	28	81	
Age			
Years	9-78	8-76	
Mean±SE	50.560± 2.773	52.185± 2.275	
FBS			
Mean±SE	92.8±1.24	160.25±59.21	<0.05
HbA1c			
Mean±SE	5.31±0.04	9.49±0.26	<0.05
Cholesterol			
Mean±SE	160.26±7.25	262.88±16.61	<0.05
Triglycerides			
Mean±SE	134.28±17.96	199.14±145.78	<0.05
LDL cholesterol			
Mean±SE	95.32±5.85	137.04±8.01	<0.05
HDL cholesterol			
Mean±SE	40.55±1.54	32.37±1.51	<0.05
AST			
Mean±SE	20.671±1.51	32.26±3.86	<0.05
ALT			
Mean±SE	21.69±2.13	26.20±3.08	<0.05
ALP			
Mean±SE	86.125±8.39	137.80±16.68	<0.05

Table 1 shows the means and standard error of all the lipid profile parameters of the participants which analyzed and it turned out that all the parameters significant difference in both diabetic and non-diabetic individuals. The findings suggest that the mean values of T-CHO (198.4), TG (316.97), and LDL-C (137.04) in diabetes patients were substantially greater than those of non-diabetic participants, which were 160.26, 134.28, and 95.32, respectively, with a P-value < 0.05. The mean HDL-C value in diabetes patients was 32.37, considerably lower than the mean value of non-diabetic participants at 40.55 (P<0.05)

In clinical practice, liver function tests (LFTs) are frequently used to screen for liver disease, track the development of established disease, and keep tabs on the effects of medicines that could be hepatotoxic. The serum aminotransferases and alkaline phosphatase are the two most prevalent LFTs. Aminotransferases, such as ALT and AST, quantify the quantity of intracellular hepatic enzymes that have entered the blood, serving as indicators of hepatocyte damage. ALP is a marker for cholestasis and biliary function. Both serum ALP and AST significantly increased in diabetic patients 137.80±16.68, 32.26±3.86 in comparison to CG 21.69±2.13 20.671±1.51 respectively (P<0.05) (Table 1). ALT level in diabetic patients was higher than in healthy individual, however there was no significant increase in patient with diabetes 26.20±3.08 compared to CG 21.69±2.13 refers to 1.2-fold increase.

3.1.Evaluation of Plt CXCL4 level in DM patients

Plasma level of CXCL4 levels increased in response to DM in diabetic patients (Figure 1A). Among 200 individuals, plasma level of CXCL4 levels measured, CXCL4 levels of 150 diabetic patients were significantly higher (15.30±1.59) compared with 50 healthy controls (6.76±1.97) (P < 0.05) (Figure 1A), refers to more than to 2-folds increase. CXCL4 levels increased by more than 2-fold in diabetic patients compared to controls (P < 0.05). Pretreatment with NSC23766 reduced CXCL4 levels by approximately 80% (P < 0.05).

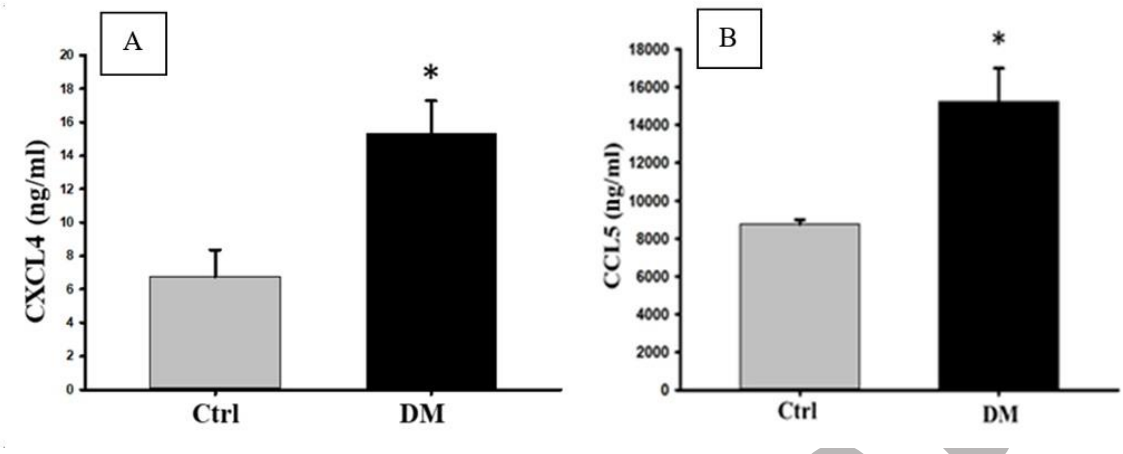

Figure 1: Activated PLTs secrete chemokines in diabetic patients. ELISA was used to quantify the levels. (**A)** CXCL4 in the diabetic patients' serum. (**B)** CCL5 in the diabetic patients' serum. Data represent mean ± SEM (Ctrl=50, DM=150).

3.2.Evaluation of Plt CCL5 level in DM patients

In response to DM, diabetic individuals' plasma levels of CCL5 increased (Figure R1B). 150 diabetes patients had significantly higher CCL5 levels (15243.56± 1748.32) than 50 healthy controls (8767.16± 232.82) of the 200 subject individuals whose plasma levels of CCL5 were tested (Figure R1B). P-value <0.05 refers to an increase in 42%.

3.3.Streptozotocin induced DM in mice

The mean FBS for the CG (sham) 85.00±6.00 mg/dl. When mice were injected with 45 mg/kg body weight with STZ, their FBS levels were considerably higher than those of the sham group ($P < 0.05$). However, the elevated blood sugar brought on by streptozotocin was significantly reduced in the group that received Rac1 inhibitor treatment. The FBS of mice was decreased from 556.2026.65 to 375.2020.7 with a P-value of 0.05 after pretreatment with 5 mg kg1 of the Rac1 inhibitor (NSC23766). Thus, NSC23766 attenuates Rac1 activation, preventing elevated BG that may trigger PLT activation.

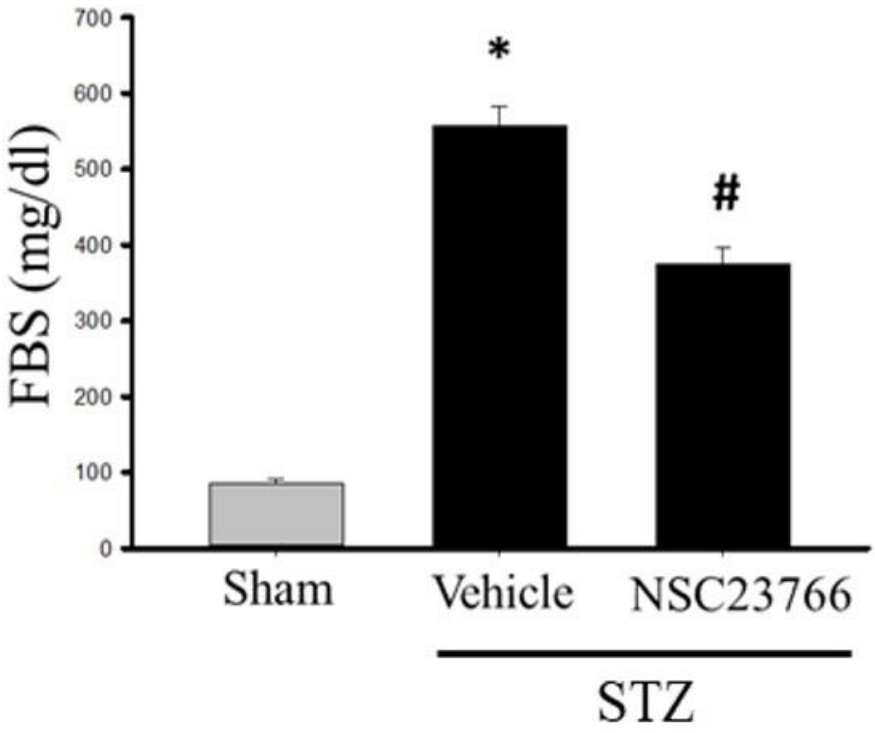

Figure 2: BG levels. Fasting BG levels were assessed on the harvest day after a 5-day induction of mice with STZ (Vehicle) and pretreatment with the Rac1 inhibitor (NSC23766). The data are shown as mean ± SEM (sham=5, vehicle=5).

3.4.Role of Rac1 in DM

Rac1 belongs to the Rho family of small GTPases, which regulates cellular growth, differentiation, migration, and inflammation. However, there is still no proof of Rac1 signaling's involvement in PLT-dependent inflammation in DM and its consequence. The study demonstrates for the first time the role of Rac1 in the release of PLT chemokines associated with DM. In order to understand Rac1's function in vehicle animals that were streptozotocin-induced, we employed the Rac1 inhibitor NSC23766. Serum from control, STZ challenge, and animals given a particular Rac 1 inhibitor was extracted for a Rac1 activation experiment in order to study how STZ activates the Rac1 GTPase. Rac1 becomes more active (GTP-binding form) after being exposed to STZ. Rac1 activation

caused by STZ was decreased by pretreatment with the Rac1 inhibitor NSC23766 (Figure 3).

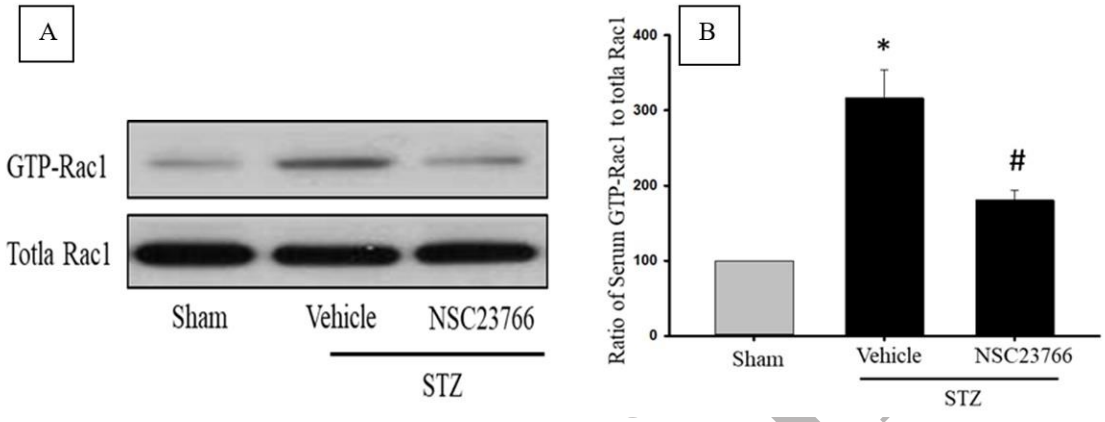

Figure 3: Rac1 activity in serum of DM-induced mice. (A) Rac1-GTP was quantified using western blotting employing GST-PAK pull-down beads upon STZ induction. (B) Band intensities were assessed using densitometry and normalized to the overall Rac1 levels. Western blots exemplify three distinct experiments. Mice received treatment with the Rac1 inhibitor NSC23766 (5 mg/kg) or vehicle administered 30 minutes before STZ induction. Sham mice functioned as negative controls. The bars indicate the mean ± SEM, with n = 3.

3.5.Rac1 regulates Plt secretion of CXCL4 in diabetic patients

In sham mice, DM elevated plasma CXCL4 levels from 6.40±0.4 ng/ml to 13.60±1.32 ng/ml, representing a 2.12-fold increase. We induced diabetes mellitus in diabetic mice by administering 45 mg/kg body weight of SZT into the plasma. This suggests that DM causes the PLT chemokine production of CXCL4. Notably, DM-induced PLT aggregation and chemokine secretion in PLTs were considerably decreased by NSC23766, a Rac1 inhibitor. This indicates that NSC23766, an efficient inhibitor of Rac1 activation, also inhibits the elevated level of PLT chemokines. NSC23766 pretreatment reduces CXCL4 serum levels in diabetic mice by more than 80%, from 13.60±1.32 ng/ml to 7.20±0.8 ng/ml (Figure 4).

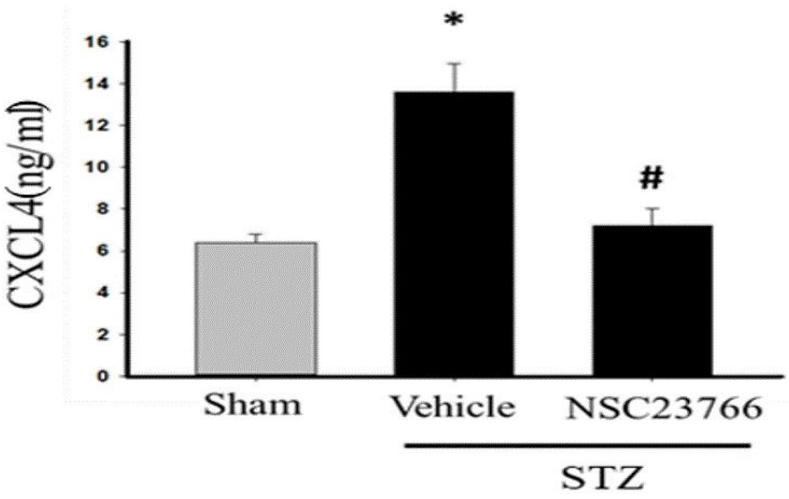

Figure 4: Activated PLTs release chemokines in diabetic mice. ELISA was used to quantify the amounts of CXCL 4 in the serum of diabetic mice. Data are presented as mean ± SEM (sham=5, vehicle=5).

3.6.Rac1 regulates PLT secretion of CCL5 in diabetic patients

Plasma CCL5 levels increased from 21.800±1.908 pg/ml in sham mice to 67.400±7.332 pg/ml due to DM, representing a threefold elevation. We found that administering SZT (45 mg/kg body weight) into plasma promotes diabetes mellitus, suggesting that diabetes stimulates the release of the chemokine CCL5 in Plts of diabetic mice. Markedly, the Rac1 inhibitor (NSC23766) greatly decreased PLT aggregation and chemokine release brought on by DM, demonstrating that NSC23766, an efficient Rac1 activation inhibitor, also inhibits the elevated level of PLT chemokines. In diabetic mice, pretreatment with NSC23766 reduced blood levels of CCL5 from 67.4±7.332 pg/ml to 30±2.236 pg/ml, a decrease of more than 55.5%. Inhibiting Rac1 may reduce CXCL4 and CCL5 levels, thereby alleviating PLT-driven inflammation, a significant factor in the onset of microvascular and macrovascular problems in diabetes (Figure 5).

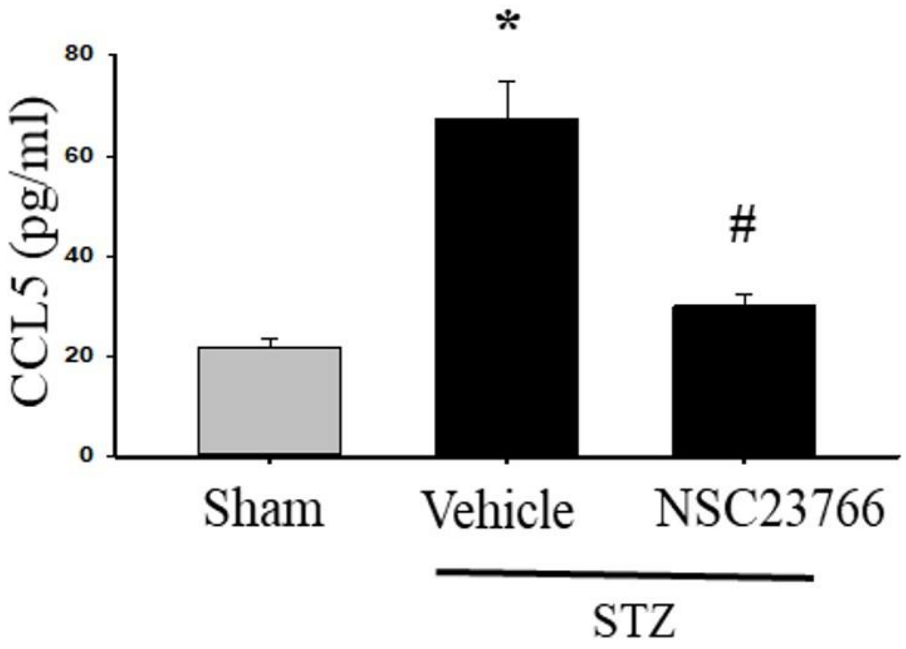

Figure 5: Activated PLTs release chemokines in diabetic mice. ELISA was used to measure the concentrations of CCL5 in the plasma of diabetic mice. The data are shown as mean ± SEM (sham=5, vehicle=5).

3.7.Histopathological alterations of kidney

While the treatment group (induced diabetes) had modest vacuolar degenerations and dispersed chronic inflammatory cell infiltration, the kidney's histopathological alteration in comparison to the normal CG revealed normal kidney histology architecture. Following therapy, there will be a decrease in both degeneration and inflammatory cells (Figure 6 B). When streptozotocin injections were used to produce diabetes mellitus, the resulting kidney damage was considerably greater in diabetic mice than in the sham group (1.13±0.08) ($P < 0.05$). Also, the mice's histology score dropped to 0.26±0.21 with a p-value <0.05 after receiving NSC23766 (Figure 6 A).

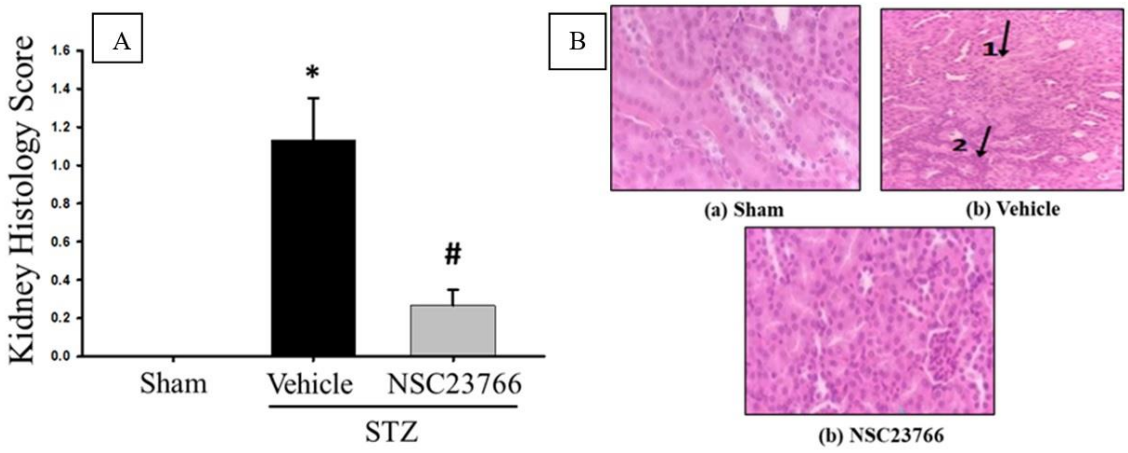

Figure 6 (A): Rac1 regulates kidney damage in DM. The kidney's histopathology score. (B): In DM, Rac1 controls kidney injury. The kidney's representative portions (H&E X100) are shown. (a) The renal histology of the CG is normal. (b) Vacuolar degeneration (arrow 2) and dispersed chronic inflammatory cells (arrow 1). (c) Inflammatory cells and vacuolar degeneration were reduced in the treatment group.

3.8.Histopathology alterations of liver

The histological examination of the normal control liver revealed intact hepatic architecture, but the treatment group (induced diabetes) exhibited vascular congestion, degenerative hepatocytes (reversible damage), and dispersed chronic inflammatory cell infiltration (lymphocytes). However, there was only minimal vascular congestion in the group that received NSC23766 prior to treatment (Figure 7 B). Our research showed that the mice with diabetes had severe liver damage. Figure 7 shows a significant increase in liver damage caused by streptozotocin injections in mice when compared to the CG (1.40.08) ($P < 0.05$). In contrast, NSC23766 decreased the mice's histology score to 0.73 ±0.06 with a P-value of less than 0.05 (Figure 7 A).

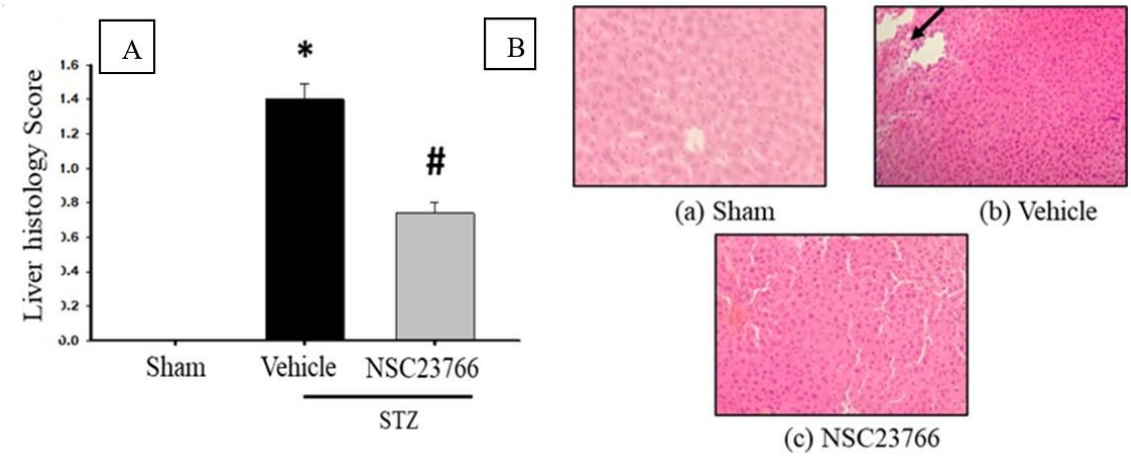

Figure 7 (A): Rac1 regulates liver damage in DM. Histology score in the liver. (B): In DM, Rac1 controls liver damage. The liver's representative H&E X100 slices are shown. (a) Normal liver histology in a healthy CG patient. (b) dispersed chronic inflammatory cell infiltration, vascular congestion, and degenerative hepatocytes as shown by the vehicle's arrow. (c) Only minimal vascular congestion was seen in the treatment group.

3.9.Histopathology alterations of lung

The histopathological examination of the normal control lung revealed a typical histological architecture, whereas the treated group (induced diabetes) exhibited pathological alterations, including moderate destruction of the alveolar structure, loss of normal lung characteristics, and moderate infiltration of mixed inflammatory cells accompanied by tissue destruction. While after giving treatment there was mild mix inflammatory cell infiltration. A substantial degree of lung injury was demonstrated by our results (Figure R8 B). Streptozotocin injection used to cause DM significantly increased the risk of lung damage in diabetic mice when compared to the CG 1.26±0.20 ($P<0.05$) (Figure 8). Morever, the mice's histological score was decreased by NSC23766 treatment to 0.38 ±0.046 ($P<0.05$) (Figure 8 A).

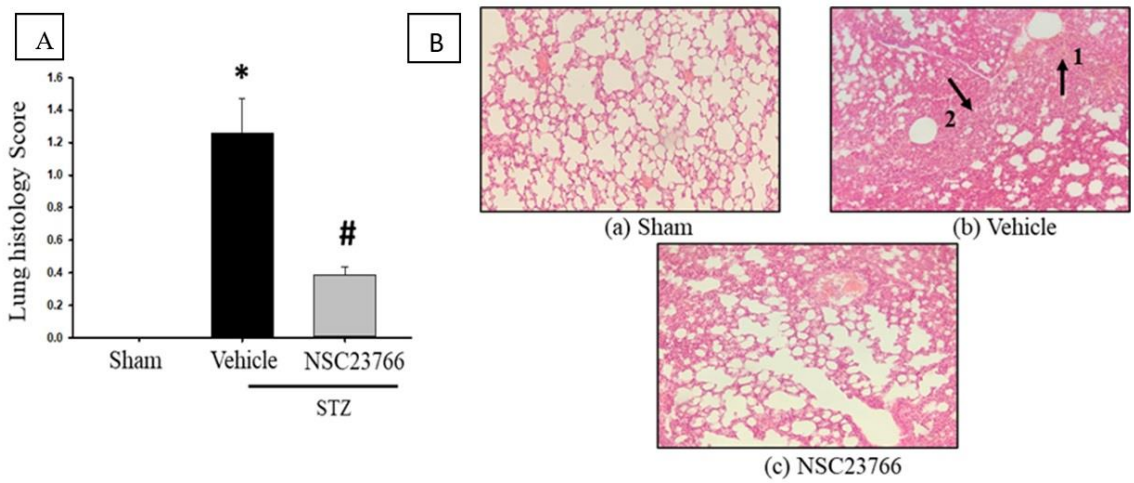

Figure 8 (A): Rac1 regulates lung damage in DM. Histology score in the lung. **(B):** Rac1 regulates lung damage in DM. Representative (H&E X100) sections of the lung are shown. (a) Normal histology of the lung of normal CG. (b) moderate destruction to the alveolar architecture, loos of lung features and moderate scattered mix inflammatory cells infiltration with tissue destruction as shown in (arrow 1 & 2). (c) Treatment group showed mild mix inflammatory cell infiltration.

3.10. Histopathology alterations of pancreas

The histopathological examination of the pancreas in the normal CG revealed typical pancreatic histological architecture, whereas the treated group exhibited vascular congestion, mild degeneration, and reversible injury to the pancreatic beta cell islets, along with scattered inflammatory cells (lymphocytes) and fibrosis (fibroblasts). While after giving treatment there was regenerative ability with the restoration of the relatively normal architecture of islets of the pancreas including beta cells (Figure R9 B). Streptozotocin injection used to cause diabetes in mice significantly increased the risk of pancreas injury by 0.83±0.14 ($P<0.05$) (Figure R9). NSC23766 treatment, however, resulted in a histological score reduction in mice of 0.32 ±0.16 with a P-value of <0.05 (Figure 9 A).

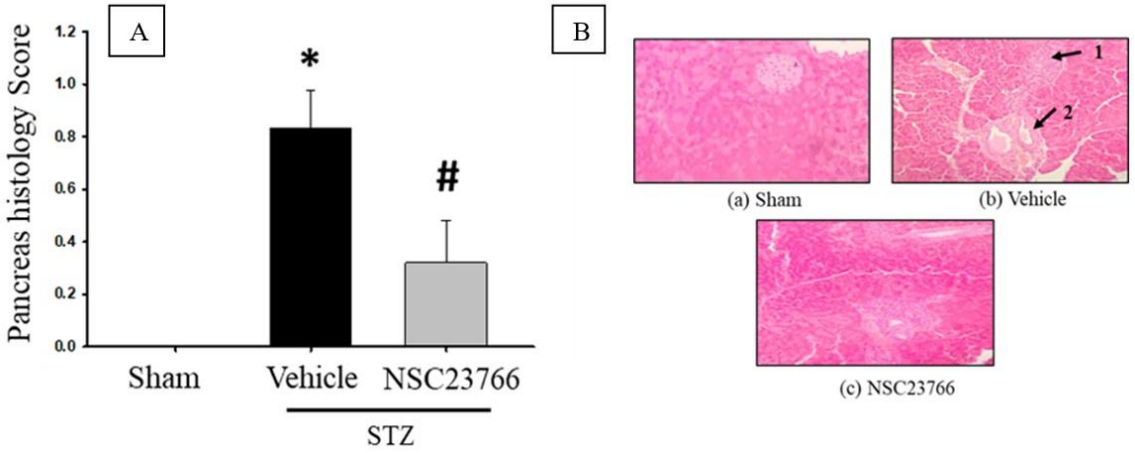

Figure 9 (A): Rac1 regulates pancreas damage in DM. Histology score in the pancreas. **(B):** Rac1 regulates pancreas damage in DM. Representative (H&E X100) sections of the pancreas are shown. (a) Normal histology of the pancreas of normal CG. (b) vascular congestion, mild degeneration and damage to the islets of pancreas beta cells as shown in (arrow 1), and scattered inflammatory cell (lymphocyte) and fibrosis (fibroblast) (arrow 2). (c) Treatment group regenerative ability with the restoration of the relatively normal architecture of islets of the pancreas including beta cells.

4. Discussion

Diabetes mellitus is a chronic metabolic condition marked by elevated blood glucose levels, potentially resulting in significant consequences that impact many organs in the body. The WHO reports a consistent rise in the global prevalence of diabetes. If this trend continues, DM is estimated that the global prevalence of diabetes will reach 439 by 2030. Chronic hyperglycemia, a defining feature of diabetes, is responsible for the development of vascular problems that contribute to increased morbidity and mortality rates (2). Furthermore, DM is a major risk factor for CVD (13). Hyperglycemia, particularly in the endothelial lining of blood vessels, increases the production of ROS, leading to vascular complications (14). In fact, vascular complications associated with diabetes are responsible for more than 75% of deaths among diabetic patients (1).

In addition to its role as a risk factor for complications, DM is also associated with a hypercoagulable state, with diabetic patients exhibiting increased PLT adhesion and aggregation (15). PMPs, which are released during PLT activation or physical stimulation, play a role in this process. PMPs consist of membranous microvesicles and fragments with coagulative activity (16). Diabetic patients, both with type 1 and T2DM, have been found to have elevated levels of cell adhesion molecules, such as PAM-1 and soluble P-selectin, further emphasizing the involvement of PLTs in diabetes-related complications (17). Additionally, macrophages and monocytes are essential for the development of microvascular problems in diabetes (18).

Chemokines, such as CXCL4 (PLT factor 4) and CCL5 (RANTES), are small cytokines that bind to specific G protein-coupled receptors (GPCRs) and regulate leukocyte activation and trafficking to inflammation sites. PLT α-granules store chemokines like CXCL4 and CCL5, which attract neutrophils and monocytes (19). These chemokines can also form heteromers, such as CXCL4-CCL5, which have a strong effect on the induction of monocytes and neutrophils (20). Dyslipidemia, which is defined by increased LDL cholesterol and triglyceride levels and reduced HDL cholesterol, is often linked to T2DM. For CAD, this dyslipidemic profile is a major risk factor (6). Dyslipidemia in T2DM is influenced by insulin resistance and abnormal lipoprotein metabolism, including impaired lipoprotein lipase (LpL) activity and altered peripheral insulin effects on muscle and adipose tissue (6). Therefore, it is important to closely monitor these patients.

The liver is significantly affected by diabetes. Specific markers of hepatic injury, such as ALT, AST, and GGT, are often elevated in diabetic individuals. Hepatocellular carcinoma and cirrhosis may arise from liver illnesses including NAFLD and NASH, which have been linked to T2DM (7). When insulin-resistant, an excess of free fatty acids may cause direct harm to liver cells via a number of pathways, such as oxidative stress, mitochondrial malfunction, and cell membrane disruption (21). The small G protein Rac1 is a crucial signaling molecule associated with diabetes, since it participates in intracellular transduction pathways that activate NADPH oxidase. Rac1 necessitates isoprenylation to transit from the cytosol to the plasma membrane, where it aids in the assembly of NADPH oxidase subunits, a vital process in the generation of ROS. Elevated

Rac1 activation may exacerbate vascular oxidative stress generated by hyperglycemia. While the function of Rac1 in PLT chemokine secretion and activation has been investigated in a number of diseases, including pancreatitis and septic lung damage, research on its activation and effects on PLT chemokines in diabetes is still continuing (22).

Inhibition of Rac1 signaling by using NSC23766 leads to ameliorate PLT activation and chemokines secretion. Rac1 signaling is elevated in active PLT and controls PLT-derived CXCL4 in DM. Rac1 also controls PLT secretion of CCL5, a powerful stimulator for neutrophil buildup in DM. Finally, Rac1 inhibition attenuates PLT activation via attenuation of chemokines secretion. Future studies should investigate the long-term effects of Rac1 inhibition on diabetic complications in clinical settings and explore potential combination therapies targeting multiple inflammatory pathways.

One limitation of the current study was the use of a single animal model, which may limit generalizability to human populations. Further research is needed to explore the precise signaling pathways through which Rac1 modulates chemokine release in diabetic conditions

In conclusion, diabetes is a complex metabolic disorder that affects multiple organ systems. PLTs, chemokines, dyslipidemia, and liver dysfunction all contribute to the pathogenesis of diabetes and its associated complications. Comprehending the molecular pathways that govern diabetes and its complications is essential for formulating successful therapy methods. Examining the function of Rac1 and PLT chemokines in diabetes may provide significant insights into the disease's causes and facilitate the creation of tailored therapeutics to avert or alleviate diabetes-related problems.

Acknowledgments

None

Authors' contributions

RH, HJ and TS Designed and collected data; RH and HJ performed laboratory experience. TS and MM analysed data. MM and RA reviewed and edited draft. All authors read and approved the final version of manuscript.

Conflict of interest

The author declares that there are no conflicts of interest..

Funding

None

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author.

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