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Effect of gallic acid on purinergic signaling in lymphocytes, platelets, and serum of diabetic rats

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ABSTRACT

Diabetes Mellitus (DM) is associated with an increased susceptibility to various infections, which might be attributed to changes in immune response owing to chronic hyperglycemia. Nucleoside triphosphate diphosphohydrolase (NTPDase), 5′-nucleotidase, and adenosine deaminase (ADA) are important enzymes involved in the generation of anti-aggregant and anti-inflammatory microenvironments. The aim of this study was to evaluate the effect of gallic acid (GA) on the hematological parameters and ectonucleotidase activities in platelets, lymphocytes, and serum of diabetic rats. Experimental rats were categorized into 4 groups: (i) control -saline, (ii) control - GA, (iii) diabetic -saline, and (iv) diabetic - GA. One week after induction of DM using streptozotocin (65 mg/kg), GA (30 mg/kg) or saline was orally administered to the rats for 21 days. Our results demonstrated that the concentration of mean corpuscular hemoglobin was decreased, whereas that of red cell distribution was increased in the diabetic group, however, GA could revert these alterations. Moreover, in diabetic rats, GA reverted the increase in ATP and ADP hydrolysis and ADA activity in lymphocytes, and it prevented the increase in NTPDase and ADA activities in platelets. A decrease in ATP hydrolysis and an increase in ADP and AMP hydrolysis were observed in the serum of diabetic rats; however, GA treatment could solely revert changes in ATP hydrolysis. Our study suggests that GA exhibits beneficial effects on immuno- and thrombo-regulatory responses in DM and that these effects may be related to the modulation of purinergic signaling.

1. Introduction

Gallic acid (GA,3,4,5-trihydroxybenzoic acid) is naturally present in grape seeds, berries, reddish fruits, onions, tea leaves (green and black), and other plants. Recently, studies on GA are gaining attention owing to various biological features of this compound such as antioxidant and anti-inflammatory properties [1,2]. Importantly, studies have demonstrated that GA exerts anti-hyperglycemic effects by decreasing the levels of glucose and glycosylated hemoglobin and increasing the insulin levels in experimental models of diabetes [3].

Diabetes mellitus (DM) is a multifactorial clinical disease associated with hyperglycemia and is considered a risk factor for many other pathologies [4,5]. Literature survey has demonstrated that components of the immune system are altered in DM. These immunological alterations include changes in lymphocyte metabolism as well as functions and altered levels of specific cytokines [6,7]. Moreover, low grade inflammation, platelet hyperactivity, endothelial dysfunction, and abnormalities in coagulation are associated to increased atherothrombotic risk in DM [7–9].

Purinergic molecules such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine act as positive or negative signals that modulate the immuno- and thrombo-regulatory responses

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[10]. The balance among ATP, ADP, and adenosine concentrations depends on the activities of ectonucleotidases present on the cell surface [11]. The conversion of ATP and ADP to adenosine monophosphate (AMP) is catalyzed by nucleoside triphosphate diphosphohydrolase 1 (NTPDase1; CD39), whereas 5′-nucleotidase (CD73) catalyzes the dephosphorylation of AMP into adenosine. Subsequently, the adenosine is degraded into inosine by the action of adenosine deaminase (ADA) [11]. NTPDase, 5′-nucleotidase, and ADA enzymes are considered to play pivotal role in the generation of anti-aggregant and immuno-suppressive microenvironments [12].

Previous studies by our research group have demonstrated alterations in the activities of NTPDase, 5′-nucleotidase, and ADA in diabetic patients as well as in experimental models of this endocrinopathy [13–17]. With respect to these studies, these ectoenzymes might be suitable therapeutic targets for the management of vascular and immunological complications associated with diabetes. Therefore, the evaluation of potent compounds that can modulate purinergic signaling, increases the possibility to develop treatment strategies that can reduce the severity of complications in this metabolic disease.

In this context, the aim of the present study was to evaluate the effects of GA treatment on hematological parameters and the NTPDase, 5′-nucleotidase, and ADA activities in lymphocytes, platelets, and serum of diabetic rats.

2. Materials and methods

2.1. Experimental model

In our study, we used adult male Wistar rats that were kept on a 12 h light/12 h dark cycle at a temperature of 22 ± 2 °C with free access to food and water. All the animal procedures were approved by the Animal Ethics Committee of the Federal University of Santa Maria (protocol number: 007/2015).

2.2. Experimental induction of diabetes

In rats, type 1 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg, diluted in 0.1 M sodium citrate buffer (pH 4.5). The control group received an equivalent dose of sodium citrate buffer. Rats administered with STZ injection received a 5% glucose solution instead of water for 24 h after diabetes induction, in order to reduce mortality due to hypoglycemic shock. To assess the blood glucose, blood samples were collected through the tail vein on the 6th day after STZ or vehicle injection. Glucose levels were determined using a portable glucometer (ADVANTAGE, Boehringer Mannheim, MO, USA). Rats with high fasting glucose (250 mg/dL) were considered diabetic and were used in our study. The glucose levels were determined on the first and last days of treatment.

2.3. Treatment with GA

The rats were randomly segregated into 4 groups for treatment: (i) control-saline, (ii) control-GA, (iii) diabetic-saline, and (iv) diabetic-GA. GA treatment was initiated 1 week after the induction of diabetes. Rats of control-saline and diabetic-saline groups were orally administered with saline solution. Rats of control-GA and diabetic-GA groups were orally administered with GA (30 mg/kg), once a day at 11:00 a.m. for 21 days (Fig. 1). The dose of GA and time of treatment were based on previous studies [2,3].

2.4. Sample preparations for biochemical tests

The rats were anaesthetized using halothane and were euthanized on the 24th day after the last day of GA treatment. Cardiac puncture was performed and blood samples were collected into tubes containing sodium citrate or ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. Lymphocytes were isolated from the blood collected into tubes with EDTA and were separated on Ficoll-Histopaque density gradients as described by Böyum [18]. The platelet-rich plasma (PRP) was prepared from the blood collected into tubes with sodium citrate according to the method established by Pilla et al. [19] and modified by Lunkes et al. [14]. Additionally, whole blood was collected into tubes without an anticoagulant system and was centrifuged for 15 min. The clot was discarded and the serum was preserved. Hematological parameters were also evaluated in whole blood with EDTA and serum.

2.5. Hematological parameter analysis

The counts of red blood cells and total leucocytes as well as hemoglobin quantification were performed using a Mindray BC 2800 VET® automatic counter. The hematocrit was determined in a microhematocrit centrifuge at a rotation speed of 19.720 g for 5 min. The total plasma proteins were measured by refractometry and the concentration value was denoted as milligrams per deciliter (mg/dL). The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by indirect calculations. The leucocyte differential test was performed on blood smears stained with Diff Quick.

2.6. NTPDase and ADA activity assays in lymphocytes

After the isolation of lymphocytes, NTPDase activity was determined using the method described by Leal et al. [20]. The reaction mixture contained 0.5 mM $CaCl_2$, 120 mM NaCl, 5 mM KCl, 6 mM glucose, and 50 mM Tris-HCl buffer (pH 8.0) at a final volume of 200 µL. Lymphocytes suspended in saline solution were added to the reaction mixture (2–4 µg of total protein) and pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of substrate (ATP or ADP) at a final concentration of 2 mM and the reaction mixture was incubated for 70 min. The reaction was stopped using (10%) trichloracetic acid (TCA). The released inorganic phosphate (Pi) concentration was estimated using malachite green as colorimetric reagent and KH_2PO_4 as the standard, by following the method described by Chan et al. [21]. The specific activity is reported as nmol Pi released/min/mg of protein.

ADA activity in lymphocytes was measured using the method described by Giusti and Gakis [22]. A reaction mixture containing lymphocytes and 21 nmol/L adenosine (pH 6.5) was incubated at 37 °C for 1 h. The reaction was terminated by the addition of nitroprusside and phenol solution. In this assay, ammonia reacts with hypochlorite and phenol to produce intense blue indophenol. The ammonia concentration is directly proportional to the absorption of indophenol at 620 nm. The specific activity was reported as units per liter (U/L). 1 U ADA is defined as the amount of enzyme required to release 1 mmol of ammonia from adenosine per minute during standard assay conditions.

2.7. NTPDase, 5′-nucleotidase, and ADA activity assays in platelets

The reaction mixture for NTPDase activity contained 5 mM $CaCl_2$, 100 mM NaCl, 4 mM KCl, 5 mM glucose, and 50 mM Tris-HCl buffer (pH 7.4) as described by Lunkes et al. [14]. The reaction mixture for AMP hydrolysis contained the aforementioned mixture except 5 mM $CaCl_2$ that was replaced by 10 mM $MgCl_2$. Platelet sample (8–12 µg of total protein) was added to the reaction mixture and was pre-incubated at 37 °C for 10 min. The reaction was initiated by the addition of ATP or ADP to obtain a final concentration of 1 mM and was incubated for 1 h. 5′-nucleotidase activity with respect to AMP hydrolysis was performed using aforementioned method and the final concentration of AMP was 2 mM. The reaction was stopped with TCA (10%) and the released Pi concentration was estimated using the method established by Chan et al. [21]. The ADA activity was evaluated according to method described by Giusti and Gakis [22].