Case Series

Effectiveness of the probiotic *Bacillus subtilis* DG101 to treat type 2 diabetes mellitus triggered by SARS-CoV-2 infection

Facundo Rodriguez Ayala[1]; Néstor Cardinali[2]; Roberto Grau[3]*

[1]*Kyojin S.A. Research and Development Department. Castellanos 1335, Rosario (2000), Argentina.*

[2]*Instituto Cardinali de Nutrición. 9 de Julio 1287, Rosario (2000), Argentina.*

[3]*Universidad Nacional de Rosario. Facultad de Ciencias Bioquímicas y Farmacéuticas. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Suipacha 531, Rosario (2000), Argentina.*

*Corresponding Author: Prof. Dr. Roberto Grau

Universidad Nacional de Rosario. Facultad de Ciencias Bioquímicas y Farmacéuticas. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Rosario, Argentina.

Email: robertograu@fulbrightmail.org.

Abstract

The spore-forming probiotic *Bacillus subtilis* DG101 have proven to be an effective adjuvant intervention in the treatment of type 2 diabetes mellitus originally refractory to conventional therapy with metformin. Since the COVID-19 is able to trigger type 1 and type 2 diabetes mellitus which may not respond to medication and / or insulin levels, we were intrigued to unveil if *B. subtilis* DG101 probiotic intervention would be able to restored the physiological level of glucose and HbA1c among patients recovered from COVID-19. Herein, we report three cases of SARS-CoV-2-triggered type 2 diabetes mellitus resistant to insulin and metformin treatment in overweight patients which successfully responded to the incorporation of the probiotic *B. subtilis* DG101 to the anti-diabetic therapy.

Keywords: SARS-CoV-2; COVID-19; Type 2 diabetes mellitus; Obesity; Insulin/adiponectin; Probiotics; *Bacillus subtilis* DG101.

Introduction

At the end of 2019, an increase in the number of cases of atypical intestinal pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan province, China. Given the rapid virus dissemination around the world, WHO on March 2020, declared COVID-19 disease, a global pandemic. At the moment, 463,891,646 cases and 6,058,277 deaths have been reported [1,2]. A high prevalence of concomitant conditions including diabetes, obesity, cardiovascular diseases, hypertension, and chronic obstructive pulmonary disease are present in people with severe COVID-19 and those who died [3]. In previous disease epidemics, a greater risk of viral infection was observed cin people with overweight or obesity [1]. Recent studies have reported that COVID-19 may trigger diabetes (type 1 and type 2) in some people because the

SARS-CoV-2 can infiltrate the insulin-producer cells (β-cells) in the pancreas but also because the coronavirus may be able to infiltrate adipose tissue and negatively affects the production of the adiponectin hormone which works synergistically with insulin to regulate blood sugar levels [4]. Worsening the situation, some studies also suggest that hyperglycemia can lead to an elevated expression of angiotensin-converting enzyme 2 (ACE2), which could contribute to worse SARS-CoV-2 infection given the pathogen's affinity for this membrane protein [5,6]. Therefore, a positive and pathological feedback loop exists between diabetes and COVID-19 [7-10]. People with COVID-19 and diabetes have a worse prognosis, most probably because of the conjunction of multiple factors: high glucose concentrations at presentation, Hb1Ac greater than 10% and diabetic ketoacidosis [11,12].

The microorganisms residing in the host gut influence the efficiency of energy extraction from ingested foods, time of food intestinal residence, mucosal immunity, intestinal permeability and systemic inflammation, all factors involved in the triggering and progression of type 2 diabetes [13,14]. Interestingly, administration of probiotic bacteria has been reported as an approach to modulate the gut flora [15,16]. The WHO defined probiotics as live microorganisms, which when administered in adequate amounts and arriving alive to their sites of action (i.e., the intestine) confer a health benefit on the host (WHO, 2002) [15,16]. We previously reported two cases of type 2 diabetes mellitus, originally refractory to metformin treatment alone, which successfully responded to probiotic *Bacillus subtilis* DG101 intervention combined with appropriate medication [17]. Here, we report three cases of COVID-19-triggered type 2 diabetes mellitus in overweight subjects which successfully responded to the administration of the anti-diabetic probiotic *B. subtilis* DG101.

Case report

Case 1

A 43-year-old and 1.60 m of height woman with overweightness (body weight = 73.7 kg, BMI = 28.8 kg/m^2 and body fat = 33.8%) with no liver or renal function complications and normal sugar blood related parameters (glycemia of 95.0 mg/dl and 5.6 % of HbA1c) is infected by SARSCoV-2 in November 2020. Two months after the onset of COVID-19 she is diagnosed with type 2 diabetes with hyperglycemia (493.0 mg/dl), and HbA1c of 13.6%. The patient was hospitalized and medicated with 2 g/day of metformin and 14.5 IU of insulin. The patient was discharged from the hospital by August 2021 cured of COVID-19 but with an insulin-resistant and metformin-insensitive diabetic state (hyperglycemia of 250.0 mg/dl and HbA1c of 11.5%). She attends to our nutrition institute and is prescribed with a daily dose of 1 ml (approximately 20 drops) of the anti-diabetic probiotic *B. subtilis* natto DG101 at a concentration of 1 x 10^8 CFU (Colony Forming Units) per ml, plus a low fat / low caloric diet based on proteins and carbohydrates of low glycemic index and 2.5 g/day of metformin, with bimonthly controls. During the period of treatment, the patient continued with normal liver and renal functions, and no other adverse events were observed. Interestingly, after 3 months of treatment with the probiotic supplement (plus metformin and the modified diet), the blood levels of glucose decreased to nearly normal values, 120 mg/dl, and the HbA1c level decreased to a normal value (6.0%) as well the glucose tolerance test after an oral challenge with 75 g of glucose was in the range of physiological values (123 mg/dl).

Case 2

An overweight 58-year-old and 1.65 m of height man (body weight of 89.0 kg, BMI of 29.7 kg/m^2 and body fat of 28.3%) with normal liver and renal functions, and blood sugar levels of 93 mg/dl and 5.8% of HbA1c is hospitalized in January 2021 with SARS-CoV-2 infection which is worsened one week after hospitalization with the triggering of a type 2 diabetes mellitus state (blood glucose levels of 250 mg/dl and OGTT of 210 mg/dl). The patient is medicated with 2 g/day of metformin. After three months he is cured from COVID-19 but his glycemia only dropped out to 170 mg/dl (HbA1c value of 8.8%) and the subject became refractory to treatment, being discharged from hospital by August 2021. The patient attends to our nutrition

institute, and is prescribed with a daily dose of 1 ml (approximately 20 drops) of the anti-diabetic probiotic *B. subtilis* natto DG101 at a concentration of 1 x 10^8 CFU (Colony Forming Units) per ml, plus a low fat / low caloric diet based on proteins and carbohydrates of low glycemic index and 2.0 g/day of metformin, with bimonthly controls. After 3 month of treatment, the blood levels of glucose and the HbA1c decreased to nearly normal values (120 mg/dl and 6.3 %, respectively) without diabetic or renal complications.

Case 3

A 58-year-old man of 1.72 m of height with overweightness (body weight of 100.7 kg, BMI of 34.8 kg/m^2 and body fat of 32.3%) and normal blood sugar levels (glycemia of 94 mg/dl and Hb1Ac of 5.7%) is infected with SARS-CoV-2 in May 2021 and is hospitalized. Two and a half months later, a type 2 diabetes state (the blood levels of glucose increased to 240 mg/dl and the levels of HbA1c raised to 8.9%) is added to his COVID-19 state, and in addition triglycerides increased from 197 mg/dl to 300 mg/dl. After 7 (seven) months, the subject was discharged from hospital by December 2021 cured of COVID-19 but with hyperglycemia (OGTT 300 mg/dl) and triglyceride levels of 297 mg/dl. The patient attends to our nutrition institute and is prescribed with a daily dose of 1 ml (approximately 20 drops) of *B. subtilis* natto DG101 at a concentration of 1 x 10^8 CFU per ml, 2 g/day of metformin plus a low fat / low caloric diet based on proteins and carbohydrates of low glycemic index, plus bimonthly controls. After 4 months of treatment with the probiotic supplement, the blood levels of glucose decreased to 110 mg/dl, the HbA1c level decreased to 6.2% and triglycerides returned to a near physiological value of 180 mg/dl.

Discusion

Previous studies have proven that the spore-forming probiotic *B. subtilis* natto DG101 is an effective intervention in the treatment of type 2 diabetes mellitus originally refractory to conventional therapy [17,18]. *B. subtilis* has the property to form resistant spores, that are 100% viable (alive) at the time of consumption, and capable of successfully traversing the acidic environment of the stomach, reaching the intestine, and germinating there to give rise to the active form of the probiotic and to an effective concentration [19,20]. Here, we used the probiotic bacterium *B. subtilis* natto DG101 [21] as a possible co-adjuvant therapy to treat diabetic people that do not respond properly to medication after suffered COVID-19. We presented three cases of type 2 diabetes mellitus with different degrees of severity in overweight patients after SARS-CoV-2 infection. The three patients showed an excellent control of their sugar-related blood parameters (glycemia, Hb1Ac and OGTT) in few months after the probiotic *B. subtilis* natto DG101 was incorporated to the patient diet and treatment (Table 1).

Probably, the anti-diabetic effect of the probiotic *B. subtilis* natto DG101 is not due to an action at pancreatic level (e.g., regulating insulin production) because the control of sugar blood levels in diabetic persons by this probiotic is not mediated by an increase in insulin production [17,18]. We hypothesize that the probiotic *B. subtilis* natto DG101 might work, directly or indirectly, influencing the cellular glucose transporters and transforming them into more sensitive or better responders to the blood circulating levels of the hormones insulin and/or adi-

Table 1: Serum concentration of glucose expressed as mg/dl and Hb1Ac expressed as % in three patients (Case 1, 2 and 3) each of one in three different stages: (a) before the patient gets sick with SARSCoV-2, (b) when the patients are diagnosed with COVID-19 and type 2 diabetes mellitus, and (c) when cured from COVID-19 but still diabetic patients are treated with the probiotic *B. subtilis* natto DG101. Data represent mean ± S.E.M. P values were calculated using repeated-measures ANOVA with Bonferroni's post hoc analysis, P < 0.05.

	Case 1			Case 2			Case 3		
	Before COVID-19 [a]	After COVID-19 [b]	After *B. subtilis* DG101 intervention [c]	Before COVID-19 [a]	After COVID-19 [b]	After *B. subtilis* DG101 intervention [c]	Before COVID-19 [a]	After COVID-19 [b]	After *B. subtilis* DG101 intervention [c]
Glycemia (mg/dl)	95 ± 4.00	493 ± 20.00	120 ± 6.10	93 ± 3.80	250 ± 11.00	120 ± 5.30	94 ± 3.85	240 ± 11.50	110 ± 5.60
Hb1Ac (%)	5.6 ± 0.24	13.6 ± 0.70	6.0 ± 0.30	5.8 ± 0.29	8.8 ± 0.48	6.3 ± 0.33	5.7 ± 0.27	8.9 ± 0.45	6.2 ± 0.31

ponectin to internalize the sugar into the cell. However, since the complexity of the diabetes disease, the exact mechanism(s) of probiotic action against type 2 diabetes mellitus have to be worked out and required intense future research. At the moment, the present results argue for the beneficial effect that probiotic *B. subtilis* natto DG101 possesses for the treatment of SARS-CoV-2-triggered or -aggravated type 2 diabetes mellitus and other diseases [22,23].

Declarations

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