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Oxidizing and reducing responses of granulocytes from type 2 diabetic patients

Maria Regina Calsolari[a], Leonardo Oliveira Medina[a], Elaine Patrícia Cunha[a], Clara Araújo Veloso[a], Miriam Martins Chaves[b], Adriana Bosco[a], José Augusto Nogueira-Machado[a,*]

[a]*Hospital Santa Casa de Misericórdia de Belo Horizonte, MG, Brazil*
[b]*Universidade Federal de Minas Gerais, MG, Brazil*

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Abstract

Oxidizing/reducing response by granulocytes and a potential correlation between reactive oxygen species generation and triglyceride, total cholesterol, low-density lipoprotein, high-density lipoprotein, body mass index, fasting plasma glucose, glycemic control (hemoglobin A_{1c}), or duration of diabetes were examined in type 2 diabetic patients and in healthy subjects. An increase in both oxidizing and reducing responses was observed in cells from diabetic patients relative to normoglycemic individuals. The increase in oxidizing response was nearly 2-fold higher, whereas the antioxidant response increased by 50%. Although reactive oxygen species generation from healthy subjects was correlated with levels of low-density lipoprotein (positive correlation), high-density lipoprotein (negative correlation), and body mass index (positive correlation), no such associations were observed in diabetic subjects, suggesting either an intrinsic perturbation in the oxidant/antioxidant response or possibly due to the effect of the various medications being taken by these patients. These issues need further study.

1. Introduction

Diabetes mellitus is associated with increase in the generation of reactive oxygen species (ROS) [1,2]. The intake of glucose induces an increase in the generation of ROS and a decrease in the levels of antioxidant reserve [2,3]. Medications used in the treatment of diabetes, such as β-blockers and statins, decrease ROS generation by leukocytes [4,5], whereas fatty acids and triglycerides can stimulate their production [6]. It has been suggested that oxidized low-density lipoprotein (LDL) may produce a pro-inflammatory action, whereas high-density lipoprotein (HDL) possesses anti-inflammatory properties [7,8]. The aim of the present work was to study the balance between ROS generation and intracellular antioxidant defenses in resting human granulocytes derived from type 2 diabetic patients and from healthy subjects and to evaluate the potential correlation between the production of ROS and the levels of total cholesterol, LDL cholesterol and HDL cholesterol, triglycerides, body mass index (BMI), fasting plasma glucose, and glycemic control (hemoglobin A_{1c} [HbA_{1c}]) in both groups.

2. Subjects and methods

The ethical committee of the Hospital Santa Casa de Belo Horizonte approved this study. Appropriate informed consent was obtained from each participant. Healthy subjects (n = 16) and individuals with type 2 diabetes mellitus (n = 27) were selected based on (i) an age of between 30 and 75 years and (ii) a fasting glucose level of more than 140 mg/dL (determined on 2 separate occasions) for the diabetic group and less than 100 mg/dL for the control group. Smokers, pregnant women, those with alcohol abuse problem, dementia, inflammation, malignant disease, fasting glucose between 110 and 140 mg/dL, or infection were excluded from the study. The average ages were 56.0 ± 13.0 and 59.6 ± 11.0 for healthy individuals and diabetic patients, respectively. Diabetic patients were

* Corresponding author. Núcleo de Pós-Graduação e Pesquisa, Hospital Santa Casa de Misericórdia de Belo Horizonte, Av. Francisco Sales, IIII, Santa Efigênia-30150-221, Belo Horizonte, MG, Brazil. Tel.: +55 31 3238 88 38; fax: +55 31 3238 88 38.
E-mail address: aunog.bh@terra.com.br (J.A. Nogueira-Machado).

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taking rosiglitazone and/or metformin or sulfonylurea, β-blockers, and statin and/or fibrate. Granulocytes were purified from 10.0-mL samples of heparinized venous blood, using the Ficoll-Hypaque gradient method described by Bicalho et al [9]. The ROS was quantified in a luminol-dependent chemiluminescence assay according to Nogueira-Machado et al [10]. The intracellular antioxidant response was performed by the quantitative MTT [3-4(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma Co, St. Louis, MO) dye reduction according to Malaquias et al [11]. Briefly, the quantitative MTT dye reduction was performed as follows: granulocytes (5×10^5/100 μL) from either diabetic patients or from healthy subjects and 25 μL of MTT (5.0 mg/mL in phosphate-buffered saline) were incubated during 120 minutes at 37°C. The reaction was stopped by adding 1.5 mL of isopropanol-HCl (0.04 N), and the absorbance was read at 570 nm. The experiments were done in triplicate. Serum levels of total cholesterol, LDL cholesterol and HDL cholesterol, triglycerides, HbA_{1c}, and plasma glucose were determined using commercial kits.

3. Results and discussion

The generation of ROS (oxidizing response) and MTT dye reduction (antioxidant response) in resting granulocytes from type 2 diabetic patients were significantly greater than that observed with granulocytes from healthy subjects. Dandona et al [12] have observed similar results in mononuclear cells from type 1 and 2 diabetic patients in relation to ROS generation. The simultaneous increase of both oxidizing and reducing responses may suggest the presence of an altered balance between the oxidizing and the intracellular reducing responses in diabetic patients, but in absence of a typical oxidative stress (Table 1). No correlations were observed between ROS production by resting granulocytes from diabetic patients and total cholesterol, LDL cholesterol or HDL cholesterol, triglycerides, BMI, fasting plasma glucose, glycemic control (HbA_{1c}), or duration of diabetes, although ROS generation was correlated positively with BMI ($r = 0.887$, $P = .001$); and positively ($r = 0.835$, $P = .031$) and negatively ($r = -0.739$, $P = .036$), respectively, with LDL cholesterol or HDL cholesterol in healthy patients. Araujo et al [6] reported a correlation between ROS generation and the levels of triglycerides, LDL, and HDL in normoglycemic patients with hyperlipidemia. The absence of correlation between ROS production and the biochemical markers of diabetic patients and the altered (increased) oxidizing/reducing balance considered in the present study could be occasioned by the medications being received by the diabetic group including statin, rosiglitazone/metformim, and β-blockers. Twelve diabetic patients were taking metformin or rosiglitazone, β-blocker, and statin. Their respective antioxidant responses were higher than the average observed in granulocytes from healthy subjects. Oxidized LDLs can exhibit a pro-inflammatory effect by modulating respiratory burst activities of granulocytes [5,13]. In contrast, HDLs are able to prevent an increase in the generation of ROS, and inhibit redox-sensitive

Table 1
Oxidizing and antioxidant responses of granulocytes and potential correlations between ROS generation and serum levels of total cholesterol, LDL cholesterol and HDL cholesterol, and triglycerides

Parameters	Healthy subjects			Type 2 diabetic patients		
	Mean[a]			Mean[b]		
Oxidizing response—ROS generation (RLU/min $\times 10^{-3}$)	28 ± 6.5	$P < .05$		53 ± 18.0		
Antioxidant response—MTT dye reduction (OD at 570 nm $\times 10^3$)	223.0 ± 20	$P < .05$		346.0 ± 40		
		Correlation with ROS			Correlation with ROS	
		Correlation coefficient (r)[c]	Significance (P)[d]		Correlation coefficient (r)[c]	Significance (P)[d]
Cholesterol (mg/dL)	195 ± 27	0.557	.152	189 ± 9.0	0.051	.878
LDL cholesterol (mg/dL)	125 ± 17.0	0.835[e]	.038	110 ± 6.0	0.150	.660
HDL cholesterol (mg/dL)	55 ± 3.0	−0.739[e]	.036	49 ± 2.5	−0.145	.653
Triglyceride (mg/dL)	198 ± 77	0.221	.599	160 ± 30	0.119	.713
Fasting plasma glucose (mg/dL)	87 ± 2	0.609	.062	170 ± 16	0.030	.886
HbA_{1c} (%)	5 ± 0.08	0.629	.181	8.7 ± 0.32	0.143	.487
BMI (kg/m^2)	24.8 ± 1.5	0.887[e]	.001	28.5 ± 1.3	0.220	.493
Duration of diabetes	–	–	–	9.4 ± 1.4	−0.257	.375

RLU indicates relative light units; OD, optical density.
[a] Values shown are means ± SD (n = 16).
[b] Values shown are means ± SD (n = 27).
[c] Pearson correlation.
[d] Student t test.
[e] Significant.

signaling and the subsequent inflammatory response [14]. Thus, the ROS/LDL and ROS/HDL associations are closely linked with inflammatory processes. Taken together, our results suggest that oxidizing/reducing responses of granulocytes and the correlation between ROS and biochemical markers need to be studied carefully because of the possibility of interference of the kind of medications being used by the patients. However, in cells from healthy subjects, these parameters studied may be used for determining a pro- or anti-inflammatory status.

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