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Research paper

Potential effects of metformin in DNA BER system based on oxidative status in type 2 diabetes

Irem Dogan Turacli [a, *], Tuba Candar [b], Emine Berrin Yuksel [c], Sebnem Kalay [d], Ali Kemal Oguz [d], Selda Demirtas [b]

[a] Medical Biology Department, Ufuk University, Ankara, 06520, Turkey
[b] Medical Biochemistry Department, Ufuk University, Ankara, 06520, Turkey
[c] Medical Genetics Department, Ufuk University, Ankara, 06520, Turkey
[d] Internal Medicine Department, Ufuk University, Ankara, 06520, Turkey

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ABSTRACT

Metformin is used to reduce hyperglycemia that induces energetic stress and leads to reduction in gluconeogenesis. Also, metformin inhibits complex I in oxidative phosphorylation, thereby decreasing cellular ATP levels. Activation of AMPK by the reduced ATP levels can induce inhibition of reactive oxygen species (ROS) production and activate p53-mediated DNA repair.

DNA polymerase-β and XRCC1 function to repair DNA damages in the BER (base excision repair) system. In type 2 diabetes patients, metformin can enhance AMPK activation therefore suppress oxidative stress. The changes on oxidative stress may alter p53's function and effect many cellular pathways such as; DNA repair. In our project we aim to understand the effects of metformin on p53 and DNA-BER system based on the oxidative status in type 2 diabetes patients.

Oxidative and antioxidative capacity, catalase, SOD, GPx activities and, DNA pol beta, XRCC1 and p53 levels were measured in metformin using or non-using type 2 diabetes patients and controls.

Metformin enhanced SOD and GPx activities in type 2 diabetes patients but the reflection of this increase to the total antioxidant capacity was not significant. Although the increase in DNA pol beta was not significant, XRCC1 and p53 levels were significantly upregulated with metformin treatment in type 2 diabetes patients. Our study reinforces the potential benefit of metformin in antioxidative capacity to protect cells from diabetic oxidative stress and in regulation of DNA BER system.

1. Introduction

Diabetes Mellitus is a multifactorial metabolic condition characterized by impaired insulin action and secretion that leads to disordered levels of serum glucose. The genetic components of this disease still need to be clarified for the prevention and pharmalogical intervention [1]. More than 90% of the diabetes patients are diagnosed with type 2 diabetes and the prevalence for type 2 diabetes is rapidly increasing on a global scale [2]. Metformin is the first-line oral glucose-lowering drug to control hyperglycemia in type 2 diabetes. It is reliable, cost effective, has broad spectrum of pleiotropic effects and good tolerability by patients. Metformin reduces glucose levels by suppression of hepatic glucose production and improves blood glucose control by enhancing insulin-stimulated glucose disposal in the peripheral tissues thus, sensitizing the tissues for insulin [3]. Also, epidemiological studies indicate that the incidence of cancer is reduced in type 2 diabetes treated with metformin [4]. These effects are mostly mediated by the activation of Liver kinase B1 (LKB-1), which directly phosphorylates and activates AMP-activated protein kinase (AMPK), a central metabolic sensor [5,6]. AMPK is a metabolic switch, which is activated when intracellular ATP levels are low and ADP levels are high. It also regulates lipid and glucose metabolism in several tissues by controlling mTOR (mammalian target of rapamycin) pathway, which controls the translation of a number of cell metabolism regulators [7].

AMPK has been shown to directly phosphorylate p53 on serine

* Corresponding author. Medical Biology Department, Faculty of Medicine, Ufuk University, Ankara, 06520, Turkey.
E-mail address: doganirem@gmail.com (I. Dogan Turacli).

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15 and this phosphorylation initiates AMPK-dependent cell-cycle arrest [8]. p53, the guardian of the genome, regulates DNA repair, senescence, apoptosis, differentiation as well as cellular responses to oxidative stresses [9]. Therefore, p53 is a highly important control point for all cellular processes. AMPK activation has been shown to reduce ROS levels and induce antioxidant status of vascular endothelial cells [10]. Metformin has antioxidant properties that reduces reactive oxygen species (ROS) by inhibiting mitochondrial oxidative phosphorylation [11].

It has been reported previously diabetes patients showed more oxidative damage to DNA, with increased generation of ROS, than controls [12–14]. Oxidative stress, hypoxia and oncogene activation can induce p53 activation. If the intensity of stress is high, p53 would act as a pro-apoptotic molecule and activate cellular death and senescence pathways. However, p53 would act as an antioxidant molecule and reduce ROS accumulation if stress is low [9] (Fig. 1). Thereby it activates DNA repair gene expressions [15]. In general, it is accepted that ROS levels will be high due to the increased intracellular stress in type 2 diabetes; Metformin would decrease ROS levels in which p53 gain antioxidant activity and stimulate cellular survival by inhibiting DNA damage [16]. In addition, it has been shown that metformin decreased p53 protein abundance depending on oxidative stress depletion [17]. Although metformin decreases oxidative stress formation, decreased oxidative stress inhibits p53 activity, which is an unintended consequence for the safety of cellular processes.

Above different DNA repair mechanisms, it is well determined how an array of DNA repair mechanisms appear to function in various DNA damages [18]. Among the DNA repair mechanisms, DNA base excision repair (BER) system is one of the most efficient repair process for correcting oxidation, alkylation, deamination, depurination/depyrimidination lesions and maintain genomic integrity. The short-patch of BER system requires several repair specific proteins and works in a multi stage model. First, DNA glycosidase recognizes the damaged region and hydrolyses the nitrogen carbon linkage between the base and deoxyribose. The resulting abasic site (AP-site) is processed by an AP-endonuclease APE-1. The gap is filled by DNA polymerase beta and XRCC1 proteins correctly. Finally, DNA ligase catalyzes the phosphodiester bond formation between the last unligated nucleotides [19,20]. It has been shown that p53 activates several DNA repair genes such as APE and DNA polymerase beta [21] [22].

In our study, we investigated how metformin effects the oxidant and antioxidant status, and depending on the oxidative stress how p53 and DNA BER system enzymes changed by metformin treatment in type 2 diabetes patients. It can be estimated that metformin treated patients have lesser oxidative stress and higher BER activity than others. The molecular action of metformin may change oxidative and antioxidative capacity. This change can transform the action of p53 between prooxidant and antioxidant status, which directed us to search its effects on BER DNA repair in type 2 diabetes.

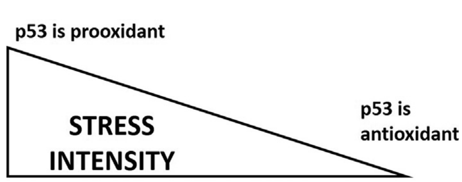

Fig. 1. The responses of p53 to the varying intensity of stress conditions. p53 can have a prooxidant role due to increased stress levels, whereas the decreased or basal stress levels may induce its antioxidant role.

2. Materials and methods

2.1. Study subjects

A total of 57 patients diagnosed with type 2 diabetes and 30 healthy controls matching age and gender proportions were selected from İnternal Medicine Department/Ufuk University. The diagnosis was in line with the diagnostic criteria and classification of diabetes of Turkish Society of Endocrinology and Metabolism. Exclusion criteria were as follows: patients suffering from acute or chronic infection; patients with liver disease; and patients that have concomitant endocrine, metabolic and renal diseases, patients or controls with familial cancer syndrome history, patients using insulin or other oral diabetic agents rather than metformin, female pregnant patients, patients or controls using antioxidant agents. Type 2 diabetes patient group was composed of newly diagnosed patients, who did not get any agent to regulate blood glucose or insulin levels. Metformin using diabetes group was composed of type 2 diabetes patients who receive 1000 mg twice daily metformin for more than 6 months. The control group was composed of individuals with no history of diabetes. The study protocol was approved by the Ethics Committee of Ufuk University School of Medicine, and complied with the guidelines and principles of the Declaration of Helsinki. The written informed consent was taken from all participants.

All the selected subjects were fasted for 8–12 h. About 5 mL of venous blood sample was collected the next morning in an anticoagulant tube at room temperature. The venous blood sample was centrifuged at 3000 rpm for 15 min. The upper supernatant was taken and aliquoted in eppendorf tubes, and frozen at −80 °C for the standby use.

2.2. Oxidative capacity detection

Reactive oxygen species levels were tested using PerOx (TOS/TOC) commercial kit (Immundiagnostik, Bensheim, Germany) by ChemWell 2910 Automated EIA and Chemistry Analyzer (Awareness Technology, FL, USA). The principle of the kit was to determine the total content of lipid peroxides in a given sample. The measurement of the oxidation of tetramethylbenzidine (TMB) into a colored product was evaluated using photometry at 450 nm.

2.3. Antioxidative capacity detection

Antioxidative capacity was tested using ImAnOx (TAS/TAC) commercial kit (Immundiagnostik, Bensheim, Germany) by ChemWell 2910 Automated EIA and Chemistry Analyzer (Awareness Technology, FL, USA). The principle of the kit to determine the antioxidative capacity of a given sample by adding defined amount of H_2O_2, which allows the elimination of a certain amount of the exogenously provided H_2O_2 by antioxidants in the sample The difference between added and remaining H_2O_2 is determined photometrically by an enzymatic reaction which involves the conversion of tetramethylbenzidine to a colored product. After the elimination reaction, the samples were measured at 450 nm.

2.4. Catalase (CAT) activity assay

Catalase activity was tested using Cayman Chemical Catalase Assay kit (Item no: 707002) (Cayman Chemical, USA) which has peroxidatic function. Catalase detoxifies H2O2 by catalyzing it to molecular oxygen and water. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured spectrophotometrically with 4-amino-3-hydrazino-5- mercapto-