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Short Communication

THE EFFECT OF MELATONIN ON ANTIOXIDANT ENZYMES IN HUMAN DIABETIC SKIN FIBROBLASTS

EWA KILAŃCZYK and MARIA BRYCZEWSKA*

Department of General Biophysics, University of Łódź, Banacha 12/16,
90-237 Łódź, Poland

Abstract: Melatonin plays several important physiological functions in mammals, such as immune enhancement and regulation of dark-light signal transduction. Melatonin is also known to be an endogenous free radical scavenger and an efficient antioxidant. It detoxifies a variety of free radicals and reactive oxygen intermediates, including the hydroxyl radical, singlet oxygen and nitric oxide. These radicals participate in many diseases, for example diabetes. This study determined the effect of melatonin on the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and the level of glutathione (GSH) in human diabetic (C2 line) skin fibroblasts.

Confluent monolayers of control (S2 line) and diabetic (C2 line) skin fibroblasts were incubated with different concentrations of melatonin: 10, 50, 100 and 1000 $\mu\text{mol/l}$ at 37°C for 24 h. Next, the GSH level and SOD, CAT and GPx activities were measured colorimetrically. The activities of the antioxidant enzymes and the GSH level were lower in diabetic skin fibroblasts than in the control S2 line. Concentrations of melatonin of 100 and 1000 $\mu\text{mol/l}$ caused a significant increase in the enzymes' activities and GSH level.

Key Words: Melatonin, Antioxidant Enzymes, Diabetes Type 2

INTRODUCTION

The pineal hormone melatonin is the mediator of external light in physiological adaptation to day and night rhythms. It is a small and highly lipophilic molecule that can easily traverse membranes and accumulate up to 30 times its

*Corresponding author: Phone/fax: +48 42 635 44 74, E-mail: marbrys@biol.uni.lodz.pl
Abbreviations used: SOD - superoxide dismutase; CAT - catalase; GPx - glutathione peroxidase; GSH - reduced glutathione; NBT - nitroblue tetrazolium; GSSG - oxidized glutathione; NADPH - nicotinamide adenine dinucleotide phosphate, reduced form.

concentration within the blood [1], thus affecting both the cytosolic and nuclear components of the cells. Melatonin also effectively reduces several direct parameters of oxidative damage. The toxicity of free radicals can be mitigated by direct free radical scavengers such as tocopherols, ascorbic acid, β -caroten and GSH and by indirect antioxidants. A number of enzymes act as indirect antioxidants since they metabolise free radicals or their reactive intermediates to harmless products. Enzymes which function in this capacity include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [2]. Melatonin has been found to be both a direct free radical scavenger and an indirect antioxidant. There is also some evidence that melatonin would protect against the hyperglycemia-induced free radicals associated with diabetes [3]. The aim of this study was to investigate whether melatonin will improve antioxidant imbalance in human diabetic skin fibroblasts.

MATERIAL AND METHODS

Control human skin fibroblasts (line S2) and diabetic type 2 fibroblasts (line C2) were obtained from The Centre of Child Health, Warsaw, (Poland). These fibroblasts were grown as monolayers in Eagle's basal medium with 10% newborn calf serum at 37°C in an atmosphere of 5% CO₂ and 95% air. The protein content was determined by the method of Lowry [4]. After incubation for 24 h with various concentrations of melatonin (10, 50, 100 and 1000 μ mol/l) the activities of the antioxidant enzymes (SOD, CAT, GPx) and the GSH level were measured.

Detection of enzymes activity

Superoxide dismutase (SOD) (U/ mg protein)

The activity of SOD was detected using the nitroblue tetrazolium (NBT) reduction method according to Beuchamp and Fridovich.[5]. A competitive inhibition was performed using a xantine/xantine oxidase system as a source of O₂^{•-} to reduce NBT at a constant rate of 0.0165 absorbance units/min as monitored spectrophotometrically at 540 nm.

Catalase (CAT) (U/ mg protein)

The activity of CAT was measured spectrophotometrically according to Beers and Sizer [6]. The absorbance was read at 210 nm.

Glutathione peroxidase (GPx) (U/ mg protein)

The activity of GPx was assayed using reduced glutathione (GSH) and *t*-butylhydroperoxide as substrates by monitoring the production of oxidized glutathione (GSSG) through NADPH oxidation. The rate of NADPH oxidation was monitored spectrophotometrically at 360 nm [7].

Glutathione (GSH) (mmol/l)

The level of GSH was determined according to the method of Ellman [8]. The concentration of GSH was monitored spectrophotometrically at 412 nm.

Statistical analysis

The results were expressed as mean \pm SD, and the statistical significance of the differences was evaluated using Student's t-test.

RESULTS AND DISCUSSION

In our work, the activities of the antioxidant enzymes SOD and CAT and the level of GSH were higher in the S2 line control skin fibroblasts than in the diabetic C2 line. The activity of GPx in the control and diabetic cell lines was similar. The activity of SOD was significantly higher for all concentrations of melatonin in comparison to the control (Fig. 1A). The concentrations of melatonin from 50 to 1000 $\mu\text{mol/l}$ caused in the diabetic C2 line a significant ($p < 0.001$) increase in: CAT activity (from 11.2 to 16.1 U/mg protein), GPx activity (from 19.2 to 23.9 U/mg protein) and GSH level (from 14.3 to 19.8 mmol GSH/mg protein) (Figs. 1 B, C, D). No changes in enzyme activities in the control S2 cell line after incubation with all the concentrations of melatonin were observed.

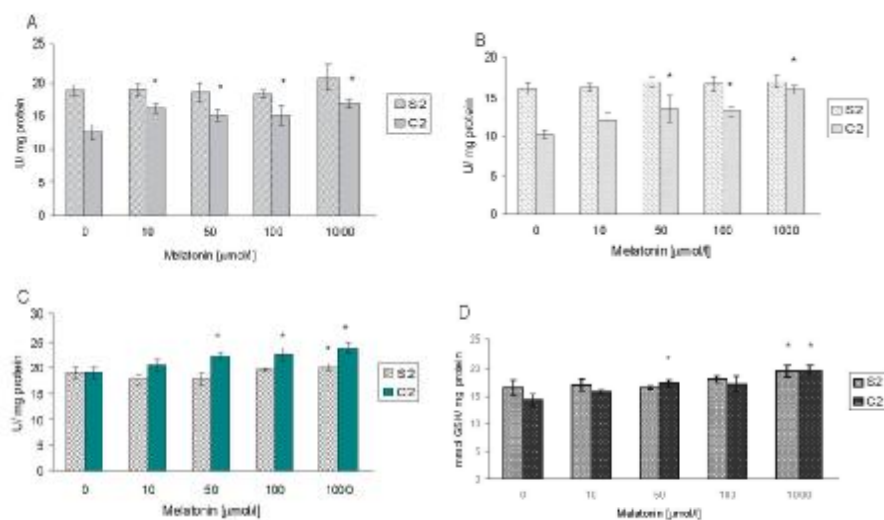


Fig. 1. The effect of melatonin on: SOD (A), CAT (B), GPx (C) activities and GSH level (D) in the control and diabetic skin fibroblasts.

A number of *in vitro* and *in vivo* experiments support a role for melatonin as a natural antioxidant molecule. This indolamine is more efficient at scavenging both hydroxy radicals and peroxy radicals than other antioxidants such as glutathione or vitamin E. In addition to its direct role as a scavenger, melatonin stimulates the level of GSH and the activity of the antioxidant enzymes glutathione peroxidase and Cu,Zn-superoxide dismutase [9, 10]. Our results confirm these data. It has been shown that melatonin increases antioxidant enzyme activity by inducing their gene expression [10]. The increase in the GSH

level induced by melatonin may function to reduce oxidative stress and to regulate cell growth.

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