

Proceedings of the
International Conference on
**ANIMAL & HEALTH
HUMAN & SAFETY**

PROCEEDINGS



- 6 - 8 December 2009
- Palm Garden IOI Resort
Putrajaya, Malaysia

Editors

M. Ariff Omar • Rasedee Abdullah
Gurmeet Kaur Dhaliwal
Chen Hui Cheng • M. Murugaiyah
Kalthum Hashim • Ooi Peck Toung



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6 - 8 December 2009

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A handwritten signature in black ink, appearing to read "Saleha Abdul Aziz".

Prof. Dr. Saleha Abdul Aziz
Chairperson, Organising Committee

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Melatonin as an Antioxidant in Experimental Diabetes Mellitus Type 1

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Abstract

The aim of this research was to study the effect of melatonin as antioxidant like in experimental diabetes mellitus type 1. Thirty rats were divided into five groups P0, P1, P2, P3, and P4. Group P0 as a negative control of diabetes were injected with normal saline 0.5 cc and group P1 (a positive control) were injected with alloxan 150 mg/kg without melatonin. Group P2, P3, and P4 received melatonin at 5 mg/kg, 10 mg/kg and 15 mg/kg, respectively, for seven days, then on the eighth day alloxan was given at 150 mg/kg to induce diabetes. Blood samples were collected four days after alloxan injection to measure the level of blood glucose and histopathological change of langerhans cells. The data was analyzed by using ANOVA and continued with Duncan's Multiple Range Test. The result of the experiment showed that melatonin (P2, P3 and P4) did not cause hyperglycemia, thus melatonin could decrease the damage to the Langerhans cells. It could be concluded that Melatonin can be used to prevent damage to pancreas from free radical in male wistar rat (*Rattus norvegicus*).

Keywords: melatonin, alloxan, hyperglycemia, Langerhans cells

Introduction

Free radical is an agent or chemical product originally produced inside the body from the result of oxidation metabolism process. Another source of free radicals formed in abundance is exposure to external hazards such as ultraviolet (UV) light, sulfur dioxide, ozone, tobacco, alcohol, wood smoke, asbestos, pesticides, solvents and radiation. Overwhelming production of free radical causes oxidative stress which in turn causes oxidative damage to cells, tissues and organs. Oxidative stress reaction can also causes benign disease, cancer, cataract, wrinkle and degenerative diseases. One of the degenerative diseases, known as a killer disease in Indonesia, is diabetes mellitus (DM). According to World Health Organization (WHO), Indonesia is the fourth country in the world with the most number of DM patients. In 2000, 5.6 billion people were infected and in 2006, it was predicted that as many as 14 billion people in Indonesia would have diabetes mellitus and the number could reach 21.3 billion by 2030 (Octoro, 2008 and Soegondo, 2007). Thus the objective of this research was to use melatonin as an antioxidant to prevent pancreatic damage caused by free radical.

Materials and Methods

An experimental study was performed using 30 male wistar rats (*Rattus norvegicus*) weighing 100 - 150 g. Diabetes mellitus was induced by the intraperitoneal injection of alloxan® (*Sigma Chemical*).

The animals were divided into five groups :

P0: NaCl 0.5cc/mice for 8 days (negative control)

P1: NaCl 0.5cc/mice for 7 days; day 8 injected with Alloxan 150mg/kg (positive control)

P2: Melatonin 5mg/kg for 7 days; day 8 injected with Alloxan 150mg/kg

P3: Melatonin 10mg/kg for 7 days; day 8 injected with Alloxan 150mg/kg

P4: Melatonin 15mg/kg for 7 days; day 8 injected with Alloxan 150mg/kg

The rats were then sacrificed 4 days after alloxan injection. The parameters measured were blood glucose and histopathological changes in the Langerhans cells.

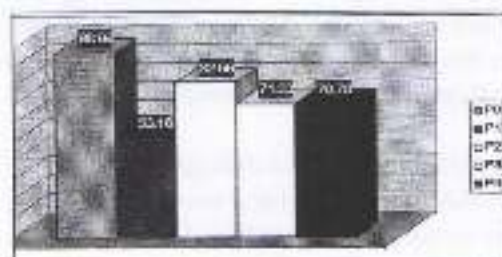


Figure1: Langerhans Cells Count (mean)

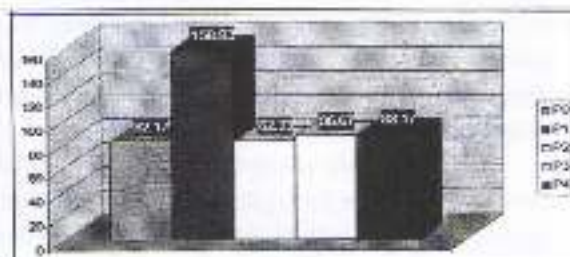


Figure 2: Blood Glucose Level (mean mg%)



Figure 3: Histopatological changes on Langerhans cells

Results

Blood glucose showed that Melatonin (Figure2) did not cause hyperglycemia in rats. Evaluation on Langerhans cells counts showed that Melatonin decreased the damage to Langerhans cells (Figure 3).

Discussion

Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells (Szudelski, 2001).

Melatonin is the main pineal gland product and it functions as a "time-giver" in the regulation of circadian rhythms. But the actions of melatonin are not restricted to the neuroendocrine physiology. In fact, it has been known as a radical scavenger, a role that has been deeply studied in all conditions where free radicals are generated. Furthermore, melatonin has been shown to act as an indirect antioxidant, since it is able to increase the activity and expression of the main antioxidant enzymes, the machinery for the glutathione synthesis, and many others direct or indirectly implicated in the free radical removal. Melatonin can also diminish the activity or expression of enzymes or factors that are considered as prooxidants (Zapico and Munteș, 2006).

It is reasonable to say that prooxidant factors are subject to rapid changes and that a lag time exists before biological systems can adapt to them. Permanent and irreversible injury, however, occurs only if the prooxidant factors are chronically higher than the maintenance and repair systems of an organism. Therefore, it is of great importance that antioxidative mechanisms, like melatonin effect, are operating throughout life. Melatonin was shown to exert a protective effect (Ozguner *et al.*, 1998). Melatonin is a highly lipophilic substance and easily penetrates the cell to stabilize lipid membrane from peroxidation and to protect the intracellular structure from damage (Jaworek *et al.*, 2007).

Melatonin has a variety of physiological, immunological, and biochemical functions. It is a direct scavenger of free radicals and has indirect antioxidant effects due to its stimulation of the expression and activity of antioxidative enzymes such as glutathione peroxidase, superoxide dismutase and catalase, and NO synthase in mammalian cells. Melatonin also reduces serum lipid levels in mammalian species and helps to prevent oxidative stress in diabetic subjects (Nishida, 2005).

All the mechanisms by which melatonin is protective of such a wide variety of molecules, i.e. lipids, proteins, DNA, etc., and in such widely diverse areas of the cell and different organs are likely not yet all identified. Suggested, but less well defined, processes which may contribute to melatonin's ability to reduce oxidative stress include stimulation of glutathione synthesis (an important antioxidant which is at high concentrations within cells), reducing electron leakage from the mitochondrial electron transport chain (which would reduce free radical generation), limiting cytokine production and inflammatory processes (actions that would also lower toxic reactant generation), and synergistic effects with other classical antioxidants (e.g. vitamins C, E and glutathione) (Reiter *et al.*, 2003).

Conclusion

It can be concluded that Melatonin can be used to prevent pancreas damage from free radical in male wistar rat (*Rattus norvegicus*) with DM disease type 1.

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