The ability of melatonin to influence oxidative stress and lower the dose of prednisolone in patients with alopecia areata

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الخلاصة

داء الحاصة البقعية من الأمراض المناعية الشائعة هو تساقط الشعر على شكل بقع دائرية ملساء ومن الممكن أن تكون غير ملحوضة.يعتبر الميلاتونين من أقوى مانعات الأكسدة المعروفة, حيث يقوم بحماية الخلايا من التأثير المحطم للجذور الحرة. يؤدي الكورتيزون المعطى عن طريق الفم إلى تحفيز نمو الشعر حيث اثبت فعليا فعالية البردنزولون لمرضى داء الحاصة البقعية مع تأثيره الجانبي المؤثر والذي يتضمن زيادة الوزن ,اضطرا بات في العمليات الأيضية ,انتشار الحبوب و اضطراب الدورة الشهرية مما يجعل صعوبة وصف الكورتزون بجرعات عالية للمرضى.

صممت هذه الدراسة لتقيم الأهمية السريرية للعلاج لمدة شهرين باستخدام مانع الأكسدة (الميلاتونين) في تقليل جرعة البردنزولون وبالتالي تقليل تأثيره الجانبي الناتج من الجرعة العالية المعطاة للمرضى المصابين بداء الحاصة البقعية.

أضهرت نتائج هذه الدراسة الأهمية السريرية العالية للميلاتونين في تقليل جرعة البردنزولون من 100 ملغ إلى 10 ملغ بين يوم و آخر وتحسين سرعة نمو الشعر عن طريق إنهاء التأثير الهدام للجذور الحرة على الجهاز المناعي و بالتالي تقليل ترسب المعقدات المناعية. وتبعا لذلك فأن هذه الدراسة تبين أهمية دور الميلاتونين في حماية الجهاز المناعي مما يؤدي إلى تقليل الحاجة إلى جرع عالية من الكورتيزونات (البردنزولون) و بالتالي تقليل التأثير الجانبي الناتج من الجرع العالية.

ABSTRACT

Alopecia areata (AA) is a common, autoimmune unpredictable, nonscaring form of hair loss. Melatonin is the most potent antioxidant known, it protect cells from so called free radical damage. Orally administered prednisone has proved effective to stimulate new hair growth for patients with AA, but its potential side effects which include weight gain, metabolic abnormalities, acne and menstrual problems makes difficulty in prescribing cortisone in high doses for patients with AA.

This study was designed to assess the clinical significance of the melatonin in reducing the dose of corticosteroids (prednisolone), and as a consequence, their side effects in patient with AA. The results of this study reveal the potential clinical significance of two months treatment with this antioxidant (melatonin) in reducing the dose of prednisolone from 100mg to 10mg administered each other day and improving the rate of hair growth by attenuating free radicals damaging effect on immune system, thereby decreasing the immune complex deposition. According to the results of this study, the use of melatonin may have an important role in protecting the immune system, and decreasing the dose and side effects that result from the use of high dose of corticosteroids.

INTRODUCTION :

Alopecia areata (AA) simply is sudden patchy hair loss. People with AA lose hair on their scalp in smooth round patches typically causing bald spots about an inch (2cm) across. The cause is unknown but it is associated with an alteration in the immunological system (1,2). Current treatment is not, at this point, directed at the etiology of alopecia areata areata but rather at the resulting inflammatory infiltrate and (presumably) the growth inhibitory factors produced by this response[1,2].

Melatonin seems to be the popular cure-all of choice at the moment. Melatonin has immunomodulating properties[3,4].

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Studies have demonstrated a stimulatory effect of melatonin on acquired immunity. A high affinity receptor binding site for melatonin has been found on T helper lymphocyte cells providing a direct link between melatonin levels and immunosensitivity. Melatonin activates these T lymphocytes into production of cytokines and this cascade of chemical signals recruits other immune cells and makes them more responsive[4].

The use of melatonin in patients with AA revealed a significant decrease in basal and hydrogen peroxide (H_2O_2) induced malondialdehyde [(MDA) (biomarker of oxidative stress)] level in erythrocytes (RBC) and plasma, increase glutathione level (major body antioxidant defence mechanism against free radical) in RBC, increase plasma total antioxidant status (TAS), increase biochemical marker level with antioxidant activity Zinc (Zn) and finally decrease copper (Cu) level[5]. These effects suggest the important role of melatonin in protecting the immune system from the oxidative damage produced by the disease and may influence the severity of the disease[5].

Research has shown that the disease responds to a variety of immunomodulating treatments, corticosteroid are part of the treatment of many disorders in which inflammation is thought to be caused by excessive or in a ppropeiate activity of the immune system like AA.

Systemic steroids are reserved for use in rapidly progressive or extensive AA[6,7,8]. A high dose up to 100 mg prednisolone daily has been recommended. In this setting acne and weight gain are commonly seen side effects[9].

This study was designed to investigate the role of melatonin in reducing the dose of prednisolone and as a consequence their side effects in patient with AA.

SUBJECTS AND METHODS:

1- subjects:

A-Controls: 20 normal controls (mean age 28.10±8.01 years) were included in this study, they were non-smokers, non-alcoholics and free from apparent other diseases.

B-Patients: 40 patients (18 females and 22 males) with AA (mean age 30.25±8.94 years) were included in this study, they were non-smokers, non-alcoholics and free from apparent other diseases with no previous treatment for AA. Patients involved in this study were under a dermatologist supervision who determined the severity of the disease according to number of the patches they have, and according to progression of disease. [Twenty of them received corticosteroids (100mg prednisolone) other day, and the other twenty receive (10mg prenisolone) other day]. Treatment schedules also included melatonin capsule 3mg each other day at night given to both groups. The treatment with melatonin for patients with AA included in this study continued for two months.

C-samples: heparinized venous blood samples were collected from patients with alopecia areata as well as from controls using plastic disposable syringes. Fresh blood sample were used for MDA and GSH measurments. Lymphocytes samples were frozen at – 20°C for up to 14 days for total antioxidant measurement(TAS).

2-methods:

- Lymphocytes and erythrocytes MDA Assay:

Measurements of lymphocytes and erythrocytes MDA (which is a by product of lipid peroxidation), based on the reaction of thiobarbituric acid (TBA) forming TBA-MDA adduct, were carried out using the modified method of Stocks and Dormandy[10] as described by Gilbert et al[11]. The results were expressed as OD/g Hb (erythrocytes) and OD/mg protein (lymphocytes).

- Lymphocytes and erythrocytes GSH assay:

Erythrocytes GSH content was determined by the method of Godin et al.[12]. Aliquots of 0.1ml packed erythrocytes were used, and combined with 0.1ml distilled water and 0.65ml of 5% TCA–1mM Na2EDTA. Then centrifuged and the supernatant analyzed for sulfhydryl group content at 412nm using 3mM DTNB in phosphate buffer. The assay mixture contained 2.6ml of 0.1M phosphate buffer (pH = 8.0), 0.3ml supernatant and 0.1ml DTNB. The light absorbance of the solution at 412nm is measured after 2 minutes waiting. Amounts of GSH were expressed as OD/g Hb.
Lymphocytes of about 0.5ml were used to assay GSH with 0.5ml 5% TCA –1mM Na2EDTA as described above Amounts of GSH were expressed as OD/mg protein.

- Total antioxidant status (TAS) determination:

TAS in lymphocytes samples was determined using a commercial kit obtained from (Randox)[13].
Assay principle: ABTS ® (2, 2-- Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the ABTS® radical. This has a relatively stable blue-green color, which is measured at 600nm. Antioxidant in the added sample causes suppression of this color production to a degree which is proportional to their concentration.

$$HX\text{-}Fe\ III + H_2O_2 \rightarrow {}^{\bullet}X\text{-}[Fe\ IV = O] + H_2O$$
$$ABTS® + X\text{-}[Fe\ IV = 0] \rightarrow ABST^{\bullet+} + HX\text{-}F\ III$$

- Zinc (Zn) and Copper (Cu) determination:

Plasma Zn and Cu were measured by flame atomic absorption spectrophotometry [(F.A.A.S) Shimadzu AA-670/GU-7]. Dilution of the plasma was made by deionized water according to the sensitivity of the (F.A.A.S) and as mentioned in the manual instruction of the manufacturer in order to avoid the viscosity and to decrease the interference of the protein[14].

The statistical significance of the difference in mean was tested by student t-test.

RESULTS:

A- Basal lymphocytes and erythrocytes MDA levels in both groups of patients were significantly higher than those in controls. Treatment with either 10 or 100mg prednisolone plus melatonin decreased MDA levels in both lymphocytes (Table-1) and erythrocytes (Table-2) as early as 1 month after treatment.
Furthermore, lymphocytes, erythrocytes GSH content and lymphocytes TAS level, was significantly lower in patients with AA compared to controls, and that treatment with both doses of prednisolone plus melatonin did significantly elevate GSH content and TAS level in patients after 1 month of treatment, and normalized these values after 2 month's of treatment (Tables-3,4,5 respectively).
On the other hand Zn level was significantly lower while Cu level higher in patients compared to controls, and that treatment with both doses of prednisolone plus melatonin did significantly elevate Zn level and decrease Cu level in patients with AA (Table-6,7).
B- Clinically there is :

1- lower incidence of prednisolone side effects (acne, weight gain and gastrointestinal disterbance) among those patients taking prednisolone dose 10mg each other day than those taking 100mg each other day (Table-8,9 and 10).
2- an obvious improvement in the rate of hair growth after one and two months treatment with antioxidant (melatonin) .

Table 1 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on lymphocytes MDA levels in patients with alopecia areata.

Group	MDA (OD/mg protein)			
	Control N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I- Melatonin+10 mg prednisolone	0.08 ± 0.17	0.16±0.03 *	0.49±0.43†	0.04±0.02†
II- Melatonin+100mg prednisolone	0.08 ± 0.17	0.23±0.17*	0.48±0.39†	0.10±0.19†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.05$).
† Significantly different from pretreatment values ($p<0.05$).
N Number of subjects

Table 2 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on erythrocytes MDA levels in patients with alopecia areata.

Group	MDA (OD /g Hb)			
	Control N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	0.20± 0.05	0.91±0.08*	0.29±0.06†	0.15±0.05†
II-Melatonin+100mg prednisolone	0.20± 0.05	0.90±0.07*	0.57±0.23†	0.29±0.14†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.05$).
† Significantly different from pretreatment values ($p<0.05$).
N Number of subjects

Table 3 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on lymphcytes glutathione levels in patients with alopecia areata.

Group	GSH (OD/mg protein)			
	Control N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	1.64±0.22	0.75±0.13*	1.14±0.15†	1.34±0.13†
II-Melatonin+100mg prednisolone	1.64±0.22	0.73±0.14*	1.07±0.14†	1.25±0.14†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.05$).
N Number of subjects

Table 4 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on erythrocytes glutathione levels in patients with alopecia areata.

Group	GSH (OD/g Hb.)			
	Control N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	19.61±5.20	12.02±1.35*	18.89±1.23†	23.14±1.53†
II-Melatonin+100mg prednisolone	19.61±5.20	11.82±0.93*	16.99±1.25†	20.78±1.93†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.01$).
† Significantly different from pretreatment values ($p<0.01$).
N Number of subjects

Table 5 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on lymphocytes TAS levels in patients with alopecia areata.

Group	TAS (µmol/mg protein)			
	Controls N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	1.16±.40	0.60±.05*	0.89± 0.06†	1.40±0.05†
II-Melatonin+100mg prednisolone	1.16±.40	0.62±0.06*	0.87±0.09†	1.33±0.11†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.05$).
† Significantly different from pretreatment values ($p<0.05$).
N Number of subjects

Table 6 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on plasma Zinc levels in patients with alopecia areata.

Group	Zinc (µg / dl)			
	Controls N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	94.35±7.29	56.45±6.28*	61.50±5.08†	67.55±5.92†
II-Melatonin+100mg prednisolone	94.35±7.29	54.55±7.90*	58.35±6.75†	63.75±5.75†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.05$).
† Significantly different from pretreatment values ($p<0.05$).
N Number of subjects

Table 7 . Effect of the addition of melatonin to prednisolone therapy (10 & 100mg) on plasma Copper levels in patients with alopecia areata.

Group	Copper (µg/dl)			
	Controls N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	76.2±11.95	92.50±4.12*	86.70±3.92†	78.50±4.03†
II-Melatonin+100mg prednisolone	76.2±11.95	93.60±4.99*	87.65±4.65†	80.35±4.59†

Values are expressed as means ± SD.
* Significantly different from control (p< 0.05).
† Significantly different from pretreatment values (p<0.05).
N Number of subjects

Table 8 . Body weight of controls and age matched patients with alopecia areata.

Group	Body weight (Kg)			
	Controls N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	69.75±17.73	70.2±13.65*	72.15±13.60†	73.80±13.52†
II-Melatonin+100mg prednisolone	69.00±11.72	69.70±12.75*	72.8±13.13†	76.20±12.32†

Values are expressed as means ± SD.
* Significantly different from control (p< 0.05).
† Significantly different from pretreatment values (p<0.05).
N Number of subjects

Table 9 . Severity of acne appearance in controls and age matched patients with alopecia areata.

Group	Presence of acne			
	Control N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	Negative	negative	+	+
II-Melatonin+100mg prednisolone	Negative	negative	++	++++

Severity of the presence of acne determined by dermalogists.
N Number of subjects

Table 10 . Severity of gastrointestinal disterbance in controls and age matched patients with alopecia areata.

Group	Presence of gastrointestinal disterbance			
	Control N=20	Patients with alopecia areata		
		Pre-treatment N=20	Months after treatment	
			1 N=20	2 N=20
I-Melatonin+10mg prednisolone	Negative	negative	+	+
II-Melatonin+100mg prednisolone	Negative	negative	++	+++

Severity of the presence of gastrointestinal disterbance determined by dermalogists.
N Number of subjects

DISCUSSION:

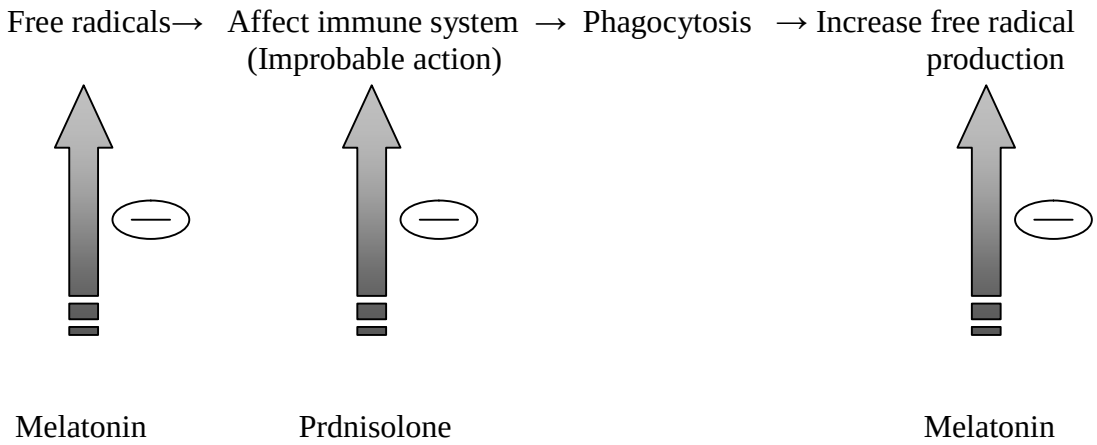
Melatonin is the most potent antioxidant known. It protects cells from free radical damage, improves and enhances the functioning of the immune system, and delays the aging process. The antioxidant effect of melatonin has been shown to be much more potent than any of the well-known antioxidants (more than twice as effective as vitamin E at scavenging peroxyl radicals)[15,16].

Corticosteroids are part of the treatment of many disorders in which inflammation is thought to be caused by excessive or inappropriate activity of the immune system like in AA[17-21]. Given in high doses, corticosteroid drugs reduce inflammation by blocking the action of prostaglandins responsible for triggering the inflammatory response[12]. They also temporarily depress the immune system by reducing the activity of certain types of white blood cells.

The present study revealed the presence of endogenous oxidative stress in both groups of patients, as manifested by the increased MDA levels in lymphocytes and erythrocytes, increase plasma Cu level and decreased GSH contents, TAS level in lymphocytes of patients with AA. This oxidative stress may result from phagocytes derived free radicals and the associated lipid peroxidation[22]. Data of the present study also indicated that, despite the difference in oral prednisolone dose (10vs. 100mg) between the two groups, addition of melatonin to prednisolone therapy resulted in comparable and significant decrease in MDA levels and correction of GSH, TAS, Zn, and Cu levels in blood, as well as similar improvement in the rate of hair growth with less side effect (acne, weight gain and gastrointestinal disturbances) regardless the dose of prednisolone. Previous study in our lab showed that, without antioxidant therapy, the effect of 100mg prednisolone was more effective than lower doses of prednisolone in improving hair growth in patients with AA[23].

This interesting and novel finding (clinical effect of melatonin, improvement in the rate of hair growth) may be due to direct and/or indirect effects of melatonin. The direct effect include its immunostimulatory and immunoenhancing effects[4,24,25] which is particularly apparent in immuno-depressive states. The immunoenhancing action of melatonin seems to be mediated by T-helper cell-derived opiod peptides as well as by lymphokines and perhaps by pituitary hormones. Melatonin-induced-immuno-opiods and lymphokines imply the presence of specific binding sites or melatonin receptors on cells on immune system. On the other hand, lymphokines such as gamma-interferon and interleukin-2 as well as thymic hormones can modulate the synthesis of melatonin in the pineal gland. The indirect effect of melatonin include its scavenging activity, which in turn decrease both oxidative damage and utilization of GSH in neutralizing phagocytes induced free radicals. So, replenishment of GSH within natural killer (immune) cells may also strengthen the immune system and increases the rate of hair growth.

Therefore, the addition of melatonin to corticosteroids attenuated the negative effects of oxidative stress on immune system and decreased the need for high dose of corticosteroid; thereby decreased the unwanted side effects associated with the prolonged use of high doses.



In conclusion, the present study presents a novel evidence for the preferential effect of the powerful antioxidant and immunostimulating hormone, melatonin when combined with traditional treatment (prednisolone) in correcting antioxidant status as well as improving the rate of hair growth in AA patients. Further studies are required to investigate the exact mechanism(s) responsible for this novel effect of melatonin.

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