

## Nocturnal urine melatonin and 6-sulphatoxymelatonin excretion at the acute stage of ischaemic stroke

**Abstract:** Melatonin's neuroprotective action has been demonstrated in experimental models of brain ischaemia. The relationship between stroke and melatonin levels has been based on scarce and small sample size studies. In addition, the changes have not been correlated with the age of patients. We compared levels of nocturnal urinary melatonin and its metabolite, 6-sulphatoxymelatonin (aMT6S) in a large series of acute ischaemic stroke patients and healthy volunteers. Consecutive ischaemic stroke patients with a first episode of anterior circulation stroke were recruited. Urine samples were collected in 127 patients on day 1 poststroke and in a control population including 216 healthy volunteers, from 20:00 to 08:00 hr. Melatonin and aMT6S were measured by radioimmunoassay. Differences in melatonin and aMT6S levels between ischaemic stroke patients and healthy volunteers were assessed by gender and age categories, using the Student's *t*-test. Melatonin excretion was decreased in stroke patients compared with healthy volunteers ( $74.1 \pm 13.9$  versus  $211.9 \pm 31.0$  ng/hr;  $P = 0.0004$ ), whereas aMT6S level was not significantly reduced ( $6371 \pm 1028$  versus  $4469 \pm 508$  ng/hr;  $P = 0.10$ ). Conversely, the stratification by age showed a significant reduction of both melatonin and aMT6S levels among ischaemic stroke patients over 70 yr ( $P = 0.001$  and  $P = 0.03$  respectively). The impact of melatonin at the acute stage of stroke on clinical severity and lesion size needs further assessment, as melatonin may have potential neuroprotective effects.

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### Introduction

Melatonin is an indole derived from serotonin and is secreted by the pineal gland; it displays a nycthemeral variation, as shown by high nocturnal and low diurnal concentrations in plasma or urine [1]. Melatonin conveys light:dark information to the organism. It plays the role of an endogenous synchronizer, able to stabilize circadian rhythms, to reinforce them and to maintain their mutual phase-relationship [2].

Melatonin's neuroprotective action has been well documented in animal experimental models of both focal and global brain ischaemia [3–5]. Neuroprotection is believed to stem from its direct free radical scavenging, indirect antioxidant activities and immunomodulatory effects [4–8]. The antioxidant defence system displays a daily rhythm which is abolished by light in humans [2]. Nocturnal urinary excretions of melatonin and 6-sulphatoxymelatonin (aMT6S), the main hepatic metabolite, display a reduction during aging [2]. In ischaemic stroke, the melatonin rhythm is impaired, with a nocturnal decrease or a tendency to phase delay [9, 10]. The potential relationship between

stroke and melatonin has based on scarce studies involving small samples of patients. In addition, stratification according to age has not been documented. We compared melatonin and aMT6S in a large series of ischaemic stroke patients and healthy volunteers.

### Patients and methods

#### Patients

Consecutive patients with a first ischaemic stroke in the anterior circulation documented by brain MRI or computerized tomography scan were recruited in the early evening from January 2007 to April 2008. Ischaemic stroke patients or their relatives provided informed consent. Exclusion criteria were posterior circulation and hemorrhagic stroke, past history of sleep disorder, significant liver dysfunction assessed by level of liver enzymes, administration of  $\beta$ -blockers which inhibit melatonin secretion, steroids and immunosuppressant agents. Control population consisted of 216 healthy drug free volunteers. They were recruited through an advertisement in the Hospices Civils de Lyon

and had given their written consent. Information on age and gender of ischaemic stroke patients and healthy volunteers are given in Table 1.

### Hormone assays

Urine samples were collected from 20:00 to 08:00 hr in healthy volunteers at home and in ischaemic stroke patients in the intensive neurological care unit. Light intensity was constantly maintained below 50 lux, in order not to inhibit melatonin secretion. We determined both melatonin and aMT6S with radioimmunoassays previously described [11, 12]. Results are expressed in ng/hr, which integrates volume and time span of urine collection. Among 216 volunteers, melatonin and aMT6S levels were assessed in 160 and 205 subjects, respectively. The missing data involved 56 and 11 healthy volunteers for melatonin and aMT6S respectively.

### Statistical analysis

All statistics were performed using SAS software version 9.1 (SPSS Inc., Paris, France). Age, available only as ordinal classes, was 'dummy' coded as 0–1, as were gender and ischaemic stroke status. Differences between ischaemic stroke patients and healthy volunteers were evaluated using chi-squared test for age and gender and the Student's *t*-test for both melatonin and aMT6S levels. Hormone data are expressed as mean  $\pm$  S.E.M. We also conducted stratified analysis by sex and age categories. Statistical significance was set at  $P < 0.05$ .

### Results

Demographic data are presented in Table 1. One-hundred and twenty-seven consecutive ischaemic stroke patients met the inclusion criteria (39 women, 88 men) and were compared with 216 healthy volunteers (123 women, 93

men). When the entire sample was considered, melatonin excretion was significantly reduced in ischaemic stroke patients compared with healthy volunteers ( $74.1 \pm 13.9$  versus  $211.9 \pm 31.0$  ng/hr;  $P = 0.0004$ ) whereas aMT6S did not show any significant decrease ( $6371 \pm 1028$  versus  $4469 \pm 508$  ng/hr;  $P = 0.10$ ).

As melatonin and aMT6S levels showed a wide distribution, the extreme values of melatonin ( $n = 35$ ) and aMT6S ( $n = 33$ ) were considered as outliers by the Tukey fence method, and thus deleted (Table 1) [13]. After exclusion of the outliers, we observed a similar significant association for melatonin and no association for aMT6S (Table 1).

The additional stratified analysis by age categories and sex showed that melatonin was significantly reduced among men ( $P < 0.0001$ ) but not in women ( $P = 0.10$ ). We observed that both melatonin and aMT6S were significantly reduced among ischaemic stroke patients, compared to healthy volunteers, over than 70 yr age ( $P = 0.0017$  and  $P = 0.03$  respectively) (Table 2).

### Discussion

Previous observations of decreased melatonin excretion in human ischaemic stroke relied mainly on a study including 13 ischaemic stroke patients and five healthy volunteers [9]. This study is the largest showing reduced melatonin levels among patients with ischaemic stroke, compared to healthy volunteers. This reduction was independent of gender. In addition, the stratified analysis by age categories showed that melatonin and aMT6S levels were significantly decreased in patients over 70 yr age. The reduction was more marked, however, for melatonin than for aMT6S levels, suggesting that melatonin determination is a more reliable marker of melatonin secretion [1]. Although an imbalance of age in the group of healthy volunteers may have hampered the

	Ischaemic stroke patients	Healthy volunteers	<i>P</i> -value
Age (years)			
18–50 (n)	28	118	<0.0001*
51–70 (n)	46	46	
> 70 (n)	53	52	
Gender			
Female (n)	39	123	<0.0001*
Male (n)	88	93	
Melatonin levels (ng/hr)			
Mean $\pm$ S.E.M.	$74.1 \pm 13.9$	$211.9 \pm 31.0$	0.0004**
Missing data	0	56	
aMT6S levels (ng/hr)			
Mean $\pm$ S.E.M.	$6371 \pm 1028$	$4469 \pm 508$	NS
Missing data	0	11	
Melatonin levels (ng/hr) <sup>a</sup>			
Mean $\pm$ S.E.M.	$48.4 \pm 4.9$	$81.1 \pm 4.2$	<0.0001**
Excluded outliers	5	30	
aMT6S levels (ng/hr) <sup>a</sup>			
Mean $\pm$ S.E.M.	$2402 \pm 233$	$2682 \pm 173$	NS
Excluded outliers	17	16	

Table 1. Demographic data and comparisons of nocturnal urinary melatonin and aMT6S levels in ischaemic stroke patients and healthy volunteers

\*Chi square test. \*\*Student's *t*-test. NS, nonsignificant.

<sup>a</sup>Levels after exclusion of the outliers using the Tukey fence.

Table 2. Stratified analysis of nocturnal urinary melatonin and aMT6s levels by gender and age categories

	Ischaemic stroke patients	Healthy volunteers	P-value
<b>Gender</b>			
Melatonin (ng/hr)			
Male	45.3 ± 5.3 (84)	87.6 ± 6.2 (65)	< 0.0001
Female	55.1 ± 10.5 (38)	74.6 ± 5.6 (65)	NS
aMT6S (ng/hr)			
Male	2417 ± 292 (78)	2430 ± 250 (87)	NS
Female	2367 ± 371 (32)	2896 ± 239 (102)	NS
<b>Age</b>			
Melatonin (ng/hr)			
18–50 years	68.5 ± 13.1 (26)	82.8 ± 5 (80)	NS
51–70 years	39.2 ± 6.8 (41)	78.3 ± 9.1 (41)	< 0.001
> 70 years	44.9 ± 7 (53)	121.2 ± 31.8 (6)	< 0.0017
aMT6S (ng/hr)			
18–50 years	3531 ± 519 (24)	2696 ± 229 (105)	NS
51–70 years	2534 ± 412(41)	2368 ± 338 (45)	NS
> 70 years	1680 ± 227 (45)	3013 ± 528 (27)	< 0.03

NS, nonsignificant; figures in brackets are the number of subjects.

interpretation of our data, the large sample allowed smoothing the analysis. The alteration of melatonin was probably not related to a direct ischaemic pineal lesion, as stroke was exclusively located within the anterior circulation, which does not supply the pineal gland. We cannot exclude, however, a direct damage of the suprachiasmatic nuclei which are supplied by deep perforators arising from the anterior circulation. Accordingly, the melatonin rhythm might be impaired due to damage to the biological clock [10].

The lower melatonin levels could be responsible for sleep and mood disorders seen in poststroke patients [9]. Environmental lighting conditions are powerful modulators of circadian rhythms, but regulation of circadian rhythms by disease states is less clear. A recent study assessed the effect of ischaemic stroke on circadian rhythms in rats using high-resolution pineal microdialysis [14]. Rats demonstrated immediate shifts in melatonin timing after stroke. In addition, melatonin rhythms displayed prolonged instability several days after stroke. Further sequential studies in human stroke are needed to determine whether the depressed levels are transitory or sustained.

Rather than melatonin secretion being impaired, melatonin levels may have been reduced due to its increased utilization as a scavenger of reactive oxygen and reactive nitrogen species [15–17] which are elevated during ischaemia/reperfusion [6, 18]. It is possible that had other urinary metabolites of melatonin been measured, which are formed when the indole functions as a scavenger, they may have been elevated [19]. This would have been especially important in this study of acute stage stroke patients as melatonin reduction in stroke may be due to this mechanism [3, 8, 19]. At this stage, we cannot assert that individuals with lower melatonin levels are more prone to stroke. The relationship between stroke severity and melatonin secretion needs further clinical and radiological evaluations. Finally, neuroprotective effects of melatonin should be assessed in randomized trials in stroke.

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