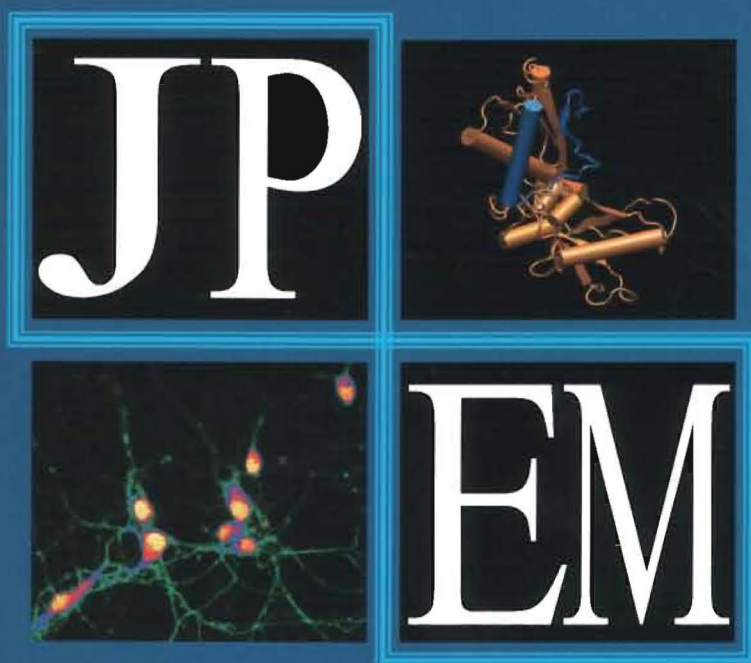


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## Melatonin and Elimination of Kynurenines in Children with Down's Syndrome

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### ABSTRACT

**Background:** Heightened activity of superoxide dimutase is an effect derived from the gene dose in the trisomy of Down's syndrome (DS), and has been related to the increased production of hydrogen peroxide and with greater lipid peroxidation. Many of the degenerative changes observed in patients with DS have been associated with the pathological effects of free radicals, and for this reason it is of interest to determine the levels present in these patients of powerful antioxidant molecules such as melatonin, and of metabolites with important neuroprotector and neurotoxic consequences such as those derived from the kynurenine pathway.

**Patients and Methods:** A study was made of 15 children with DS, together with a control group of 15 non-DS children, matched for age and sex, examined at the Hospital Costa del Sol, Marbella, Spain. Serum melatonin and serotonin were analyzed by RIA; urinary tryptophan metabolites (kynurenine pathway) were determined during periods of light and darkness (09.00-21.00 h and 21.00-9.00 h) by thin-layer chromatography.

**Results:** The mean values of serotonin and melatonin were found to be lower in the patients with DS, although the level of nocturnal secretion of melatonin was higher. Urinary excretion of kynurenine was lower in the patients with DS, although greater quantities of kynurenic acid and anthranilic acid were excreted.

**Conclusions:** Patients with DS present levels of plasma melatonin and urinary kynurenine that are lower than the corresponding levels in the control population, together with higher values of kynurenic acid and anthranilic acid. These circumstances constitute an added risk to these patients of damage by free radicals.

### KEY WORDS

Down's syndrome, melatonin, kynurenine, tryptophan

### INTRODUCTION

Down's syndrome (DS) is the commonest form of chromosome disorder and an important cause of mental handicap and premature development of neurocerebral pathological changes similar to those observed in other neurodegenerative processes that occur during adult life<sup>1,2</sup>. This fact has led some authors<sup>3</sup> to consider that oxidative processes and the generation of free radicals might play a role in such changes<sup>4,5</sup>. An effect derived from the gene load in trisomy 21 is the 1.5-fold increase in superoxide dismutase enzyme activity, with no equivalent increase in catalase activity<sup>6</sup>. In consequence, there is an accumulation of hydrogen peroxide, which is responsible, in turn, for increased lipid peroxidation. This latter process has been associated with the reduction in neurotransmitter synthesis in the central nervous system of patients with DS<sup>7</sup>. Various studies<sup>8,9</sup> have related the neurodegenerative changes observed in these pathologies to alterations in tryptophan metabolic pathways. Tryptophan can be metabolized via diverse metabolic pathways, including those of kynurenine, hydroxyindoles, methoxyindoles and indoles. In quantitative terms, the most important

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pathway of metabolic degradation is that of kynurenine, which is initiated as a break caused by the oxidative mechanism of the tryptophan pyrrole ring, with the final metabolite being L-kynurenine. It has been suggested<sup>8</sup> that the generation of nicotinamide and the restoration and maintenance of levels of nicotinamide adenine dinucleotide (NAD) are the main objectives of the metabolism of tryptophan via kynurenines. From this standpoint, the kynurenine metabolic pathway would play a fundamentally neuroprotective role, and only the accumulation of certain intermediate metabolites (quinolinic acid and 3-hydroxyanthranilic acid) would produce neurotoxic effects.

The hydroxyindole pathway metabolizes 2-5% of dietary tryptophan, and is responsible for the formation of both serotonin and melatonin, its methylate derivative. The concentration of free tryptophan is a limiting factor in the control of serotonin synthesis in the brain<sup>10</sup>. Melatonin (*N*-acetyl-5-methoxytryptamin) can also be synthesized from tryptophan via the methoxyindole pathway, which is mainly pineal. The rhythm of melatonin secretion is characterized by the existence of a peak of pineal secretion during the night, while plasma melatonin levels fall during the day. These levels measured during daylight hours are assumed to be of extrapineal secretion<sup>11,12</sup>. The observation<sup>13</sup> that melatonin and its metabolites may increase the enzyme activity of superoxide dismutase may be of particular importance for patients with DS, among whom the levels of superoxide dismutase are already above normal, due to the effect of the gene load.

The present study was designed with the following aims: a) to determine the levels of melatonin and serotonin secretion during daylight and darkness in patients with DS; b) to determine the excretory rhythm of the metabolites of tryptophan metabolism via the kynurenine pathway in patients with DS.

#### PATIENTS AND METHODS

In order to constitute the study group, we contacted the *Serranía de Ronda* Down Syndrome Association (Málaga province, Spain).

Having communicated the aims of the study, we were informed that there were 46 persons with DS in the area, of whom 22 were younger than 14 years; seven of these (31%) declined to participate in the study. Informed consent was obtained from the children's parents or guardians, and the study protocol was approved by the Hospital Ethics Committee. The control group, constituting 15 children of equivalent ages and sexes to the DS group, was recruited from children in the same geographic area, treated for other severe complaints at the Emergency Department of the *Hospital Costa del Sol* in Marbella (Málaga province, Spain). Table 1 shows the characteristics of the patients with SD and controls.

Parameters for analysis were recorded during the day (at 09.00 h) and at night (01.00 h). For this purpose, patients were asked to attend the child endocrinology clinic at the "San Cecilio" Hospital in Granada, where, after a physical examination, they were informed of the need for them to remain in the hospital under observation for 24 hours, and this request was accepted in every case. Blood samples were taken at 09.00 and 01.00 h, and urine was collected over two periods: during the day (09.00-21.00 h) and during the night (21.00-9.00 h). For all the daytime analyses, measurements included serum melatonin content, serotonin and urinary metabolites of tryptophan via the kynurenine metabolic pathway, i.e. kynurenine, 3-OH-kynurenine, kynurenic acid and anthranilic acid. For the nighttime analyses, only melatonin and the tryptophan metabolites of the kynurenine pathway were recorded.

Melatonin levels were determined by radioimmunoassay (RIA) using a Human Melatonin RIA Kit (Alpco Diagnostics, Schönenbuch, Switzerland)<sup>14</sup>. Serotonin levels were determined by RIA using a Serotonin <sup>125</sup>I-RIA kit for quantitative determination (Immuno-Biological Laboratories, Inc., Minneapolis, MN, USA)<sup>14</sup>. The urinary metabolites of tryptophan from the kynurenine pathway were determined by thin-layer chromatography, following the technique described by Coppini *et al.*<sup>15</sup>.

Statistical analysis was performed by comparison of the means for independent samples, correlation analysis and simple linear regression,

**TABLE 1**  
Characteristics of the patients and controls included in the study

|                               | Controls<br>(n = 15) | Down's syndrome<br>(n = 15) |
|-------------------------------|----------------------|-----------------------------|
| <b>Children/girls</b>         | 8/7                  | 8/7                         |
| <b>Age (months)</b>           | 59.7 (SD 29)         | 68.4 (SD 47)                |
| <b>Body weight (kg)</b>       | 21.2 (SD 7.9)        | 18.5 (SD 10.9)              |
| <b>Height (cm)</b>            | 104.5 (SD 15.2)      | 99.6 (SD 25.5)              |
| <b>BMI (kg/m<sup>2</sup>)</b> | 18.7 (SD 2.6)        | 16.6 (SD 3.1)               |

**TABLE 2**

Mean values (standard deviation) for levels of melatonin and serotonin in serum, measured during the day and during the night, among controls and patients with DS. Also shown are the mean values (standard deviation) of the urinary metabolites of the kynurenine pathway during periods of light (09.00-21.00 h) and darkness (21.00-9.00 h)

| Group                    | Controls<br>(n = 15) |              | Down's syndrome<br>(n = 15) |                 |                 |
|--------------------------|----------------------|--------------|-----------------------------|-----------------|-----------------|
|                          | Hour                 | 09.00        | 01.00                       | 09.00           | 01.00           |
| Melatonin (pg/ml)        |                      | 31.4 (9.7)   | 75.2 (31.7)                 | 22.4 (8.8) *    | 38.4 (12.3) *** |
| Serotonin (ng/ml)        |                      | 232.6 (52.6) | –                           | 92.7 (27.4) *** | –               |
| Group                    | Hour                 | 09.00-21.00  | 21.00-9.00                  | 09.00-21.00     | 21.00-9.00      |
| Kynurenin (µg/ml)        |                      | 5.6 (2.1)    | 4.2 (1.48)                  | 3.9 (1.2) *     | 3.1 (1.1) *     |
| 3-OH-Kynurenin (µg/ml)   |                      | 14.2 (6.5)   | 9.2 (5.4)                   | 15.4 (5.6)      | 11.2 (4.8)      |
| Kynurenic acid (µg/ml)   |                      | 17.6 (6.7)   | 14 (5.9)                    | 80.8 (24.6) *** | 58.2 (16) ***   |
| Xanturenic acid (µg/ml)  |                      | 13.6 (5.1)   | 10.2 (4.1)                  | 9.8 (3.3) *     | 9.1 (5.3)       |
| Anthranilic acid (µg/ml) |                      | 2.1 (1)      | 2.1 (0.9)                   | 10.9 (6.3) ***  | 7.2 (3.2) ***   |

\* p < 0.05; \*\*\* p < 0.001.

using the statistical software SPSS 14.0.

## RESULTS

It can be seen in Table 2 that the mean daytime values for serotonin were higher in the control group than in the DS group, with differences that were statistically significant. This was also true for the mean values of melatonin during the daytime, while maximal secretion values were recorded during the night. Urinary

kynurenine metabolites, during both the day and the night, were lower in the patients with DS than in the control group. The levels of 3-OH-kynurenine did not present any significant differences between the control group and the DS group, during either the day or the night. Kynurenic acid and anthranilic acid were the only metabolites of those studied that were excreted in greater quantities in the urine of the patients with DS, during both the day and the night. The values recorded for xanthurenic acid were slightly lower in the patients with DS during the daytime, while

the two groups produced similar values of this compound for the night-time samples.

There was a strong relationship between the daytime levels of melatonin and serotonin, in both the control group ( $r = 0.97$ ;  $p < 0.001$ ;  $y = -4.2 + 0.15x$ ), and the DS group ( $r = 0.94$ ;  $p < 0.001$ ;  $y = -1.34 + 0.22x$ ).

## DISCUSSION

Patients with DS presented a baseline level of melatonin and serotonin that was significantly lower than that observed in the control group; there was a circadian rhythm, but its range, again, was considerably less than that produced in the control group. In the patients with DS, the elimination of tryptophan metabolites by the kynurenine pathway was greater during daylight hours, with the highest values recorded for kynurenic acid and anthranilic acid.

In patients with DS after a tryptophan overload, the excretion of metabolites by the kynurenine pathway increases to a greater extent than in persons with no chromosomal disorder<sup>16</sup>. The pattern of elimination of tryptophan metabolites by the kynurenic acid pathway or by that of anthranilic acid, in patients with DS, is very different from that observed in healthy individuals. The lower levels reported among patients with DS of the enzyme 3-hydroxykynurenine transaminase would account for the decrease observed in the synthesis of xanthurenic acid<sup>16</sup>.

Kynurenic acid and the intermediate metabolites of the kynurenine metabolic pathways are capable of selectively activating a subgroup of dependent neural glutamate receptors (*N*-methyl-D-aspartate [NMDA]). In situations producing brain damage, such as hypoxia or neurodegenerative disease, there is a massive release of glutamate onto neurons and glia, and it has been suggested that high neural concentrations of glutamate may be toxic. The tryptophan metabolic pathway, via kynurenine, may generate metabolites that activate NMDA receptors (quinolinic acid) or that block them (kynurenic acid)<sup>9</sup>. In another study<sup>17</sup>, it was reported that melatonin may reduce the neurotoxic effects of quinolinic acid, and this aspect is relevant to the

present study, in view of the fact that patients with DS present lower levels of melatonin than controls. The present study reports significantly higher levels of urinary kynurenic acid in patients with DS, during both the day and the night; this finding leads us to consider that in patients with DS one would not expect to observe any toxic neural effect caused by hyperstimulation of the NMDA glutamate receptors.

Another metabolite, 3-OH-kynurenine, is also neurotoxic. In this case, the neural damage caused seems to be mediated by free radicals, and there is no intervention by glutamate receptors, as occurs with quinolinic acid<sup>18</sup>. There must be penetration of 3-OH-kynurenine into the cells for damage to be caused, and thus the competitive blocking of its cell transport by a supplementary dose of neutral amino acids could prevent neural damage<sup>19</sup>. It is highly probable that the toxic effect attributed to 3-OH-kynurenine is caused by its metabolite 3-hydroxyanthranilic acid, which undergoes faster processes of self-oxidation and generation of the superoxide anion. In our sample, the levels of anthranilic acid in patients with DS were significantly high, and thus these patients are likely to suffer additional harm from free radicals.

Observations made of healthy individuals<sup>20,21</sup> indicate that the pattern of tryptophan metabolism varies between the day and the night. During the day, the kynurenine pathway is strengthened, while during the night there is a greater activation of methoxyindoles and melatonin secretion. Reiter *et al.*<sup>22</sup> studied the urinary excretion of 6-OH-melatonin sulphate in 12 patients with DS, and observed the normal pattern of low daytime levels and high night-time levels in 10 patients, while in the remaining two, there was no discernible pattern. These data suggest that the neuroendocrine mechanisms regulating the secretion rate of melatonin are mainly preserved in patients with DS, although from the results derived from our study, the amplitude of the melatonin secretion peak is smaller in patients with DS than in the control group.

The correlation study carried out in our sample group showed that children with DS maintain the same ratio between melatonin and serotonin as is found among healthy individuals; this finding

should be considered a logical one, in view of the functional aspects relating the two molecules. Serotonin plays a role in modulating the central nervous system, and is involved in states of wakefulness; thus, it is of particular interest to determine serotonin levels in patients with DS. Various authors<sup>23,24</sup> have reported that the highest concentration of serotonin found in the central nervous system is within the pineal gland, and that most of it functions as a precursor for the formation of melatonin. The production of melatonin is induced during the darkness following sunset, by the intrapineal neural secretion of norepinephrine, which interacts with the alpha- and beta-adrenergic receptors located in the pinealocyte membranes, enhancing the intracellular cAMP that stimulates *N*-acetyltransferase, a limiting enzyme in the conversion of serotonin to melatonin. Melatonin and its metabolites are significant components in the body's antioxidant system<sup>13,25,26</sup>, and its importance as a scavenger of -OH free radicals has been demonstrated; it protects against lipid peroxidation and damage to DNA caused by radiolysis of cell water<sup>27</sup>. The antioxidant effect is enhanced via stimulation of peroxide glutathione activity and the inhibition of the pro-oxidative synthetase of nitric oxide in the brain<sup>22,28</sup>.

Our observations lead us to conclude that patients with DS present lower levels of plasma melatonin and urinary kynurenine than are found in the control population, with higher values of kynurenic acid and anthranilic acid. This leads to patients with DS being at added risk of neural damage from free radicals.

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