

Expression of melatonin in platelets of patients with aspirin-induced asthma

Helen V. Evsyukova

Department of Hospital Therapy, Medical Faculty, St Petersburg State University, St Petersburg, Russia

ABSTRACT

Background The diffuse neuroimmunoendocrine system (DNIES) is a universal system of response, control and organism protection. Platelets are cells of DNIES producing and storing melatonin which plays an important role in the regulation of physiological processes in the human body, under normal conditions and in pathology. Our previous study has revealed a low basic melatonin production in patients with aspirin-induced asthma (AIA). It has been suggested that low daytime production of melatonin in patients with AIA is attributable to the reduction in its synthesis in platelets in this group of patients. The objective of this study was to investigate this hypothesis.

Materials and methods The melatonin expression in platelets has been studied by means of indirect immunofluorescence in nine patients with AIA and 14 healthy subjects.

Results The results of the study have revealed that only $13.1 \pm 1.3\%$ of platelets in patients with AIA have shown melatonin-specific luminescence, compared to $97.7 \pm 0.6\%$ of platelets in healthy subjects ($P < 0.001$). No melatonin expression has been observed in the rest of platelets in patients with AIA. No significant difference between the degree of the melatonin luminescence in platelets of the patients with AIA and control group has been found.

Conclusions It can be concluded that the reduced melatonin synthesis in platelets of patients with AIA may determine a low daytime melatonin production and may lead to impairments in platelet receptors and ion channels. This results in disturbances in calcium homeostasis, which may be a cause of platelet activation and pathological response to exogenous melatonin and acetylsalicylic acid.

Keywords Aspirin-induced asthma, melatonin, neuroimmunoendocrinology, platelets.

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Introduction

Platelets play an important role in pathogenesis of aspirin-induced asthma (AIA). It has been reported that platelets isolated from these patients exhibit an abnormal response to acetylsalicylic acid (ASA) *in vitro* compared with normal individuals or allergic aspirin-tolerant asthmatics (ATA) generating cytotoxic mediators and oxygen-derived free radicals in the presence of ASA or other nonsteroidal anti-inflammatory drugs (NSAIDs) [1].

It is known that platelets are a major reservoir of serotonin (5-HT) in the human body and are capable to convert 5-HT into melatonin because they possess the enzymatic equipment necessary for this biosynthesis [2–4]. As one of the melatonin metabolites is a substance (*N*-acetyl-5-methoxy-kynurenamine) with a chemical structure similar to that of ASA [5], we have studied melatonin production in patients with AIA. Our previ-

ous studies have revealed a lower level of daytime urinary excretion of 6-sulphatoxy-melatonin, a major metabolite of melatonin, in patients with AIA than in patients with ATA and control subjects [6]. Aspirin-tolerant patients did not differ from healthy subjects by the urinary excretion of 6-sulphatoxy-melatonin. It has also been found that the platelet reaction to further addition of melatonin *in vitro* in patients with AIA differed from that of patients with ATA and healthy subjects by an increased intensity and the rate of the first phase of the ADP-induced platelet aggregation. It may be attributable to the opening of the same receptor-operated channels for calcium or/and Ca^{2+} mobilization from intracellular stores. The results of our study have allowed us to postulate that the decrease in diurnal melatonin production in patients with AIA results in a reduction in its metabolite – *N*-acetyl-5-methoxy-kynurenamine. We

suggested that a higher sensitivity of platelet reception and a distorted reaction, not only to melatonin but also to its metabolite, chemically similar to ASA, may contribute to the development of ASA symptoms after aspirin challenge during the day and increase the severity of the disease, despite the avoidance of aspirin and cross-reactive drugs [6]. The decrease in melatonin production in patients with AIA may be a reason for the overproduction of cysteinyl leukotrienes in this group of patients owing to the weakened melatonin control of 5-lipoxygenase, because there is experimental evidence for direct melatonin control of transcription of the RZR/melatonin-responding gene of 5-lipoxygenase, the key enzyme of leukotriene synthesis [7,8]. As the synthesis of melatonin in the pineal cells is known to be dramatically reduced by light, the daytime melatonin production in humans largely depends on the activity of melatonin-producing cells of the universal diffuse neuroendocrine system, which includes a large number of nonendocrine cells, especially platelets [9,10]. We have hypothesized that a low daytime production of melatonin in patients with AIA is attributable to the reduction in melatonin synthesis in platelets in this group of patients.

There are no data on melatonin production in platelets of patients with AIA. The aim of this study was to evaluate the melatonin expression in platelets of patients with AIA and to compare it with the same of healthy subjects.

Materials and methods

Subjects

Nine patients with AIA (nine women, aged from 30 to 59) have been examined. The patients with AIA have shown a clear clinical history of one or more asthma attacks precipitated by NSAIDs. Our main goal has been to determine whether the ingestion of aspirin has triggered an asthmatic response. For this reason, we have included in the main analysis only those studies in which patients have undergone provocation challenges. The analysis has demonstrated a positive aspirin-induced asthma response as a 20% or more reduction in forced expiratory volume in one second within 3 or 4 h of the challenge [11]. The study of melatonin expression in platelets was carried out while patients with asthma were in a stable clinical condition and their forced expiratory volume in 1 s (FEV₁) was $\geq 75\%$ of the predicted volume at entry to the study. None of the patients was receiving oral steroids. Five patients with AIA were receiving inhaled adrenergic agents (salbutamol or fenoterol), eight patients were treated with inhaled corticosteroids (daily dose of budesonide ≤ 800 μg). The patients did not take any cyclooxygenase inhibitor for at least 10 days before this study.

A control group of 14 healthy volunteers were recruited (12 women and two men), aged from 22 to 58. Individuals with chronic disease or any kind of clinically detectable inflamma-

tion at the time of recruitment were excluded from the study. None of the healthy volunteers was receiving any treatment.

This study was approved by the local ethics committee, and patients signed an informed consent form.

Immunohistochemistry

To verify the presence of melatonin in platelets, blood samples were extracted at 10.00–11.00 h. Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 200 g for 5 min; then, PRP was centrifuged at 800 \times g for 20 min to obtain platelet suspension. Platelet smears were prepared from the suspension by placing a drop of the suspension on an object glass followed by drying at room temperature. The dried smears were washed with saline adjusted to pH = 7.2. After another drying at room temperature, the smears were fixed with 96% ethanol for 1 min.

Melatonin in platelets was determined by indirect immunofluorescence. Platelet smears were treated with melatonin-specific antiserum (CID tech Res. Inc., Toronto, ON, Canada), kept in a chamber in damp conditions for 30 min and then washed with buffered saline and dried at room temperature. After that, the smears were treated with rabbit fluorescent isothiocyanate-conjugated serum, kept in the damp chamber for 30 min, washed with buffered saline and dried at room temperature.

Image analysis

The preparations were examined through an Axiostar-2 luminescent microscope (Zeiss, Munich, Germany). The analysis of the images obtained by microscopy was carried out with a Nikon system using the Vidotest-4 program (Videotest, Saint-Petersburg, Russia). The mean optical density of platelet luminescence was evaluated in five randomized vision fields and recorded as arbitrary units (A.U.) of melatonin expression. Moreover, the quantity of luminous platelets was counted in both groups. All image analysis and measurements were performed blind by one observer.

Reporting of the study conforms to STROBE statement for cohort studies.

Statistical data analysis

Statistical significance was assessed using Student's nonpaired *t*-test. $P < 0.05$ was accepted as significant, and $P < 0.001$ as highly significant. Results are expressed as mean \pm SEM. Data were analysed with the SPSS statistical package (IBM, NY, USA).

Results

The individual indices of the quantity of luminous platelets in patients with AIA are presented in Table 1. One can see that patients with AIA had a low quantity of luminous platelets, in

Table 1 Individual indices of the quantity of luminous platelets in patients with aspirin-induced asthma (AIA)

No.	Patients with AIA, n/N (%)	Control subjects, n/N (%)
1	7/38 (18.4 ± 6.3)	28/30 (93.3 ± 4.6)
2	10/48 (20.8 ± 5.9)	14/14 (100.0 ± 0.0)
3	11/74 (14.9 ± 4.1)	50/52 (96.1 ± 2.7)
4	8/84 (9.5 ± 3.2)	27/27 (100.0 ± 0.0)
5	6/38 (15.8 ± 5.9)	38/38 (100.0 ± 0.0)
6	29/141 (20.6 ± 3.4)	39/39 (100.0 ± 0.0)
7	6/87 (6.9 ± 2.7)	33/33 (100.0 ± 0.0)
8	3/50 (6.0 ± 3.4)	52/52 (100.0 ± 0.0)
9	5/90 (5.6 ± 2.4)	48/48 (100.0 ± 0.0)
10		32/34 (94.1 ± 4.0)
11		44/44 (100.0 ± 0.0)
12		30/31 (96.8 ± 3.2)
13		32/35 (91.4 ± 4.7)
14		36/38 (94.7 ± 3.6)

Data are presented as n/N (%) – a ratio of luminous platelets to the total quantity of platelets.

contrast to the control subjects. In patients with AIA, only $13.1 \pm 1.3\%$ of platelets showed melatonin-specific luminescence, but in healthy subjects, it was observed in $97.7 \pm 0.6\%$ of platelets ($t = 59.1$, $P < 0.001$). There was no melatonin expression in the rest of platelets of patients with AIA. As shown in Fig. 1a, the greater part of platelets from patient with AIA did not have melatonin-specific luminescence, while all platelets from healthy subject (Fig. 1b) had the expression

of melatonin. The degree of melatonin expression in luminous platelets of patients with AIA was 2.14 ± 0.02 A.U. and did not differ from that of healthy subjects (2.15 ± 0.01 A.U., $t = 0.45$, $P > 0.05$).

Discussion

The present study provides the first ever evidence that platelet melatonin production is actually absent in patients with AIA. In view of our previous results [6], we believe that the decrease in the diurnal production of melatonin in patients with AIA is attributable to a low serotonin uptake by platelets and, as a consequence, a disturbance of melatonin synthesis in platelets of patients with AIA. Our suggestion is consistent with the data obtained by Malmgren *et al.* [12], which show a low capacity of platelets for serotonin accumulation and preservation in patients with AIA.

It is known that the membrane receptor complex is a universal primary responsive system, which is largely dependent on microviscosity of membrane lipids [13]. An increased rigidity of membrane phospholipids' structures, which may be caused by the deficiency of antioxidant defence and accumulation of lipid peroxidation products, results in disturbance of the exposition of membrane receptors and thus may alter platelet responsiveness to the inducers of their aggregation [14]. Melatonin, by virtue of its antioxidant properties, can decrease protein damage by free radicals as well as increase platelets membrane microfluidity [15,16]. Low platelet melatonin generation in patients with AIA probably attenuates antioxidant defence, which may lead to an impairment in receptors and ion channels. This results in disturbances in calcium homeostasis, which in their turn may cause the platelet activation, elevation of the Ca^{2+} response to the minimal dose of melatonin *in vitro* in patients with AIA and striking deviations of platelet shape and ultrastructure found in patients with AIA [6,17]. A low

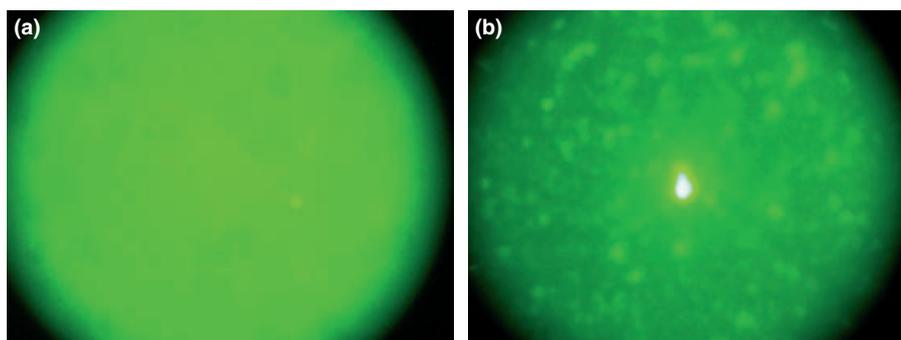


Figure 1 Immunohistochemistry showing the absence of melatonin expression in platelets from patient with aspirin-induced asthma (a) in comparison with the great number of luminous platelets from healthy subject (b).

platelet generation of melatonin in patients with AIA may also result in increased lipid peroxidation in platelets, which is consistent with published data on reduced platelet glutathione peroxidase activity and increased reactive oxygen species production in patients with AIA [18–21].

Thus, taking into account that platelets are one of the main extrapineal sources of melatonin in human organism, the defect of their membrane receptor organization resulting in lower production of melatonin in AIA provides another evidence of a major role of platelets in this form of bronchial asthma.

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Address

Department of Hospital Therapy, Medical Faculty of St Petersburg State University, Raevsky st. 7, appt.7, 194064, St Petersburg, Russia (H. V. Evsyukova).

Correspondence to: Professor Helen V. Evsyukova, Department of Hospital Therapy, Medical Faculty of St Petersburg State University, Raevsky st. 7, appt.7, 194064, St Petersburg, Russia. Tel.: 7 812 5525052; fax: 7 812 328 2361; e-mail: eevs@yandex.ru

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References

- 1 Ameisen JC, Capron A, Joseph M, Maclouf J, Vorng H, Pancre V *et al.* Aspirin-sensitive asthma: abnormal platelet response to drugs inducing asthmatic attacks. Diagnostic and physiopathological implications. *Int Arch Allergy Appl Immunol* 1985;**78**:438–48.
- 2 Champier J, Claustrat B, Besancon R, Eymin C, Killer C, Jouvet A *et al.* Evidence for tryptophan hydroxylase and hydroxy-indol-O-methyl-transferase mRNAs in human blood platelets. *Life Sci* 1997;**60**:2191–7.
- 3 Launay JM, Lamaitre BJ, Husson HP, Dreux C, Hartmann L, Da Prada M. Melatonin synthesis by rabbit platelets. *Life Sci* 1982;**31**:1487–94.
- 4 Morera AL, Abreu P. Existence of melatonin in human platelets. *J Pineal Res* 2005;**39**:432–3.
- 5 Kelly RW, Amato F, Seamark RF. N-acetyl-5-methoxy-kynurenamine, a brain metabolite of melatonin, is a potent inhibitor of prostaglandin biosynthesis. *Biochem Biophys Res Commun* 1984;**121**:372–9.
- 6 Evsyukova HV. The role of melatonin in pathogenesis of aspirin-sensitive asthma. *Eur J Clin Invest* 1999;**29**:563–7.
- 7 Carlberg C, Wiesenberg I. The orphan receptor family RZR/ROR, melatonin and 5-lipoxygenase: an unexpected relationship. *J Pineal Res* 1995;**18**:171–8.
- 8 Uz T, Longone P, Manev H. Increased hippocampal 5-lipoxygenase mRNA content in melatonin-deficient, pinealectomized rats. *J Neurochem* 1997;**69**:2220–3.
- 9 Kvetnoy IM. Extrapineal melatonin: location and role within diffuse neuroendocrine system. *Histochem J* 1999;**31**:1–12.
- 10 Pandi-Perumal SR, Srinivasan V, Maestroni GJM, Cardinali DP, Poeggeler B, Hardeland R. Melatonin: nature's most versatile biological signal? *FEBS J* 2006;**273**:2813–38.
- 11 Nizankowska E, Bestynska-Krypel A, Cmiel A, Szczeklik A. Oral and bronchial provocation tests with aspirin for diagnosis of aspirin-induced asthma. *Eur Respir J* 2000;**15**:863–9.
- 12 Malmgren R, Olsson P, Tornling G, Unge G. Acetylsalicylic asthma and migracine. A defect in serotonin (5HT) uptake in platelets. *Thromb Res* 1978;**13**:1137–9.
- 13 Usoltseva NV, Usoltsev VA. The liquid crystal state of biological structures as an essential factor of metabolism. In: *Liquid Crystals*. Ivanovo State University; 1978: 11–34 (In Russian).
- 14 Mikhailidis DP, Jeremy JY. Editorial: platelet function – the role of essential fatty acids and eicosanoids. *Prostaglandins Leukot Essent Fatty Acids* 1989;**35**:187–8.
- 15 Daniels WM, van Rensburg SJ, van Zyl JM, van der Walt BJ, Taljaard JJ. Free radical scavenging effects of melatonin and serotonin: possible mechanism. *Neuroreport* 1996;**7**:1593–6.
- 16 Reiter RJ, Tan D-X, Cabrera J, D'Arpa D, Sainz RM, Mayo JC *et al.* The oxidant/antioxidant network: role of melatonin. *Biol Signals Recept* 1999;**8**:56–63.
- 17 Aliyeva KM. *Impairments in Platelet Morphology and Functions and Their Correction with Platelet Transfusion in Atopic and Aspirin-Induced Asthma Patients*. MD Theses, Moscow; 1992: pp 25 (In Russian).
- 18 Joseph M. The generation of free radicals by blood platelets. In: Joseph M, editor. *Immunopharmacology of Platelets*. Acad. Press Lim.: London–New York; 1995: pp 209–25.
- 19 Malmgren R, Unge G, Zetterstrom O, Theorell H, de Wahl K. Lowered glutathione-peroxidase activity in asthmatic patients with food and aspirin intolerance. *Allergy* 1986;**41**:43–5.
- 20 Pearson J, Suarez-Mendez J. Abnormal platelet hydrogen peroxide metabolism in aspirin hypersensitivity. *Clin Exp Allergy* 1990;**20**:157–63.
- 21 Plaza V, Prat J, Roselló J, Ballester E, Ramis I, Mullol J *et al.* *In vitro* release of arachidonic acid metabolites, glutathione peroxidase, and oxygen-free radicals from platelets of asthmatic patients with and without aspirin intolerance. *Thorax* 1995;**50**:490–6.