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Melatonin reduces lung oxidative stress in patients with chronic obstructive pulmonary disease: a randomized, double-blind, placebo-controlled study

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Abstract

Abstract: Chronic obstructive pulmonary disease (COPD), a major cause of death and disability, is attributed to an abnormal inflammatory response by the lungs to noxious substances, primarily from cigarette smoke. Although oxidative stress is regarded as central to the pathogenesis of COPD, very few studies have examined the effects of antioxidants in this condition. This was a randomized, double-blind, placebo-controlled study on the effects of melatonin in COPD. Thirty-six consecutive patients with clinically stable moderate to very severe COPD (30 men; mean ± S.D. = 66.6 ± 7.8 yr) were randomized to receive 3 mg melatonin (N = 18) or placebo for 3 months. Oxidative stress was evaluated by 8-isoprostane levels in exhaled breath condensate at baseline (T0) and after one (T1), two (T2), and three months (T3) of treatment. Additionally, exhaled breath condensate levels of IL-8, dyspnea severity (Medical Research Council scale), lung function (spirometry), and functional exercise capacity (six min walk test) were compared at baseline and after treatment. Patients receiving melatonin showed a decrease in 8-isoprostane (T0: mean ± S.E.M. = 20.41 ± 2.92 pg/mL;

T1: 18.56 ± 2.68 pg/mL; T2: 12.68 ± 2.04 pg/mL; T3: 12.70 ± 2.18 pg/mL; $P = 0.04$; repeated measures ANOVA) with significant differences from baseline after 2 ($P = 0.03$) and 3 months ($P = 0.01$). Dyspnea was improved by melatonin ($P = 0.01$), despite no significant changes in lung function or exercise capacity. Placebo-treated patients, but not those who were given melatonin, showed an increase in IL-8 ($P = 0.03$). In summary, melatonin administration reduced oxidative stress and improved dyspnea in COPD. Further studies are necessary to determine the potential role for melatonin in the long-term management of these patients.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of death, disability, and impaired quality of life, with global social and economic repercussions. It has been characterized as a chronic progressive airflow limitation owing to an abnormal inflammatory response by the lungs to inhaled noxious gases and particles, primarily from cigarette smoke [1]. COPD is a heterogeneous condition with great patient-to-patient variation. Pathological findings usually include chronic inflammation and structural changes secondary to repeated injury and repair, which may variably affect proximal airways (chronic bronchitis), peripheral airways (obstructive bronchiolitis), lung parenchyma (emphysema), and pulmonary vasculature [2]. Mucus hypersecretion, airflow limitation, lung hyperinflation, abnormal gas exchange, and cor pulmonale are among the most significant manifestations. Systemic features of COPD, such as skeletal muscle dysfunction, cachexia, anemia, heart disease, osteopenia, and depression are common, particularly in more advanced cases, and could be related to ‘spill-over’ of inflammatory mediators into the circulation [3].

Oxidative stress is regarded as central to the pathogenesis of COPD, and increased levels of oxidative stress markers are usually present in the exhaled breath condensate, sputum, and plasma of patients with COPD [4, 5]. Oxidative stress has been shown not only to cause direct damage to lung structures but also to trigger or exacerbate other disease mechanisms in COPD, such as inflammation, protease–antiprotease imbalance, and increased apoptosis of the epithelial and endothelial cells of the lungs [6, 7]. In COPD, inflammation is poorly responsive to corticosteroid treatment, in contrast to asthma, and this has been linked to oxidative degradation induced by reactive species of histone deacetylase 2, an enzyme that promotes the compaction of chromatin, thus affecting the transcription of proinflammatory proteins [8].

Bronchodilators are currently the main therapy for patients with COPD, despite showing little or no effect on disease progression. Surprisingly, very few clinical studies have looked into the potential benefits of antioxidants in these individuals. N-acetylcysteine, a glutathione precursor, has been reported to reduce oxidative stress markers in the exhaled breath condensate of patients with COPD [9, 10]. However, its prolonged administration was unable to prevent the deterioration of pulmonary function [11].

Melatonin is a widely distributed and functionally diverse molecule [12]. In addition to its role in sleep and circadian synchronization, melatonin has pronounced antioxidant activity as it is a major scavenger of free radicals and increases antioxidative enzyme activities [13–16]. There are many reports from clinical trials showing the beneficial effects of exogenous melatonin in the prevention of cell damage in acute conditions, such as sepsis and asphyxia in newborns, as well as chronic metabolic and neurodegenerative diseases, inflammation, and cancer [17–19]. Importantly, melatonin administration is generally considered to be safe and well tolerated [20, 21]. In view of this evidence, we hypothesized that melatonin could be useful for the reduction in oxidative stress in COPD.

The main purpose of this study was to investigate the effect of exogenous melatonin on lung oxidative stress in patients with COPD. Additionally, the impact of melatonin treatment on inflammation, dyspnea, pulmonary function, and functional exercise capacity was assessed.

Material and methods

Subjects

Thirty-six consecutive patients of both genders with a previous diagnosis of COPD (GOLD stages II–IV) regularly attending a COPD Outpatient Clinic at the University Hospital, at the Federal University of Ceara, Brazil, were enrolled into the study. Patients with a history of disease exacerbation and/or use of systemic steroids within the previous 4 wk; hospitalization in the previous 8 wk; serious comorbidities, including mental disability, diabetes mellitus, renal, hepatic or congestive heart failure; alcohol or drug abuse; or who were unwilling to participate were not included in the study. The research protocol was approved by the local Research Ethics Committee (No 154/07), and written informed consent was obtained in all cases.

Study design

This was a randomized, double-blind, parallel-group, placebo-controlled study of individuals with clinically stable COPD, with a 2-wk run-in and a 3-month treatment period. Initially, patients were submitted to a medical evaluation when a brief medical history, smoking history, and medication were recorded. Patients were then asked to stop the use of inhaled steroids or any other lung medication, except for inhaled long-acting bronchodilators (Formoterol 12 mcg b.i.d. and/or Tiotropium bromide 18 mcg q.d.) or short-acting $beta_2$-agonists (salbutamol or fenoterol on demand). After the 2-wk run-in period (T0), exhaled breath condensate was collected as detailed below to determine 8-isoprostane levels; severity of dyspnea was assessed by the Medical Research Council (MRC) scale; lung function was measured by spirometry; and functional exercise capacity was determined by the six-mintue walk test (6-MWT). Patients were then randomly allocated into the melatonin or placebo groups. A block randomization process was carried out to ensure an equal proportion of drug and placebo for every four patients. Fast-release 3 mg melatonin or placebo was supplied in identical capsules to be taken in a single dose for 3 month, 2 hr before bedtime. Throughout the treatment period, patients were contacted by telephone, once every 2 wk to check for adverse effects, compliance, and exacerbation of symptoms. Exhaled breath condensate collection was repeated in all subjects at the end of the first (T1), second (T2), and third (T3) month for determination of 8-isoprostane level. Severity of dyspnea, lung function, and functional exercise capacity measurements were repeated at the end of treatment for comparison. Additionally, in a subgroup of 20 patients (10 from each group), interleukin-8 (IL-8) was measured in the exhaled breath condensate at baseline and at the end of treatment period. Patients and investigators were unaware of treatment allocation at all times.

Measurements

Collection of exhaled breath condensate was performed at baseline (T0) and at the end of the first, second, and third months (T1, T2, and T3, respectively), between 08:00 and 10:00 hr, while patients were wearing a noseclip and sitting comfortably for about 20 min Samples with an average volume of 1.5 mL were collected into a vial using a custom-made device (Fig. 1) and stored at −80°C for posterior analysis.

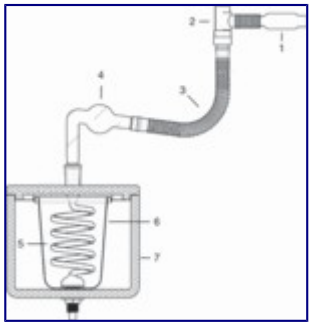

Figure 1. Systematic diagram of the exhaled breath condensate collecting system used in the study. Patients were asked to breathe through a mouthpiece (1) connected by a flow-directing device (2) to a corrugated pipe (3) which was attached to a glass spiral (5) immersed in icy water which was kept in a plastic recipient (6) inside a thermal box (7). A trapping bulb (4) was placed between the flow-directing device and the condensing tube to prevent saliva contamination of the samples.

8-Isoprostane levels in exhaled breath condensate were measured using a specific enzyme immunoassay kit (ACE EIAs; Cayman Chemicals, Ann Arbor, MI, USA) as described previously [22]. Briefly, this assay is based on the competition between 8-isoprostane and an 8-isoprostane-acetylcholinesterase conjugate for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of color is proportional to the amount of 8-isoprostane tracer bound to the well, which is inversely proportional to the amount of free 8-isoprostane. The results were expressed in pg/mL. IL-8 level in exhaled breath condensate samples were determined by commercially available Enzyme Immune Assay kit (EIA; RayBiotech, Norcross, GA, USA), according to the manufacturer's instruction.

Lung function was assessed by spirometry (Jaeger v4.31; Jaeger, Würzburg, Germany) according to published recommendation [23]. Measurements were carried out between 8 and 10 hr in all cases.

Severity of dyspnea was assessed by the MRC scale, a validated, ordinal scale based on degrees of various activities that may precipitate dyspnea. Scores range from 0 (only gets breathless with strenuous exercise) to 4 (too breathless to leave the house or becomes breathless when dressing or undressing) [24].

Functional exercise capacity was assessed by the 6-MWT. This is a practical standardized simple test that measures the distance a patient can quickly walk on flat surface in a period of 6 min [25].

Outcome measures

The primary outcome was 8-isoprostane level in exhaled breath condensate. Secondary measures were exhaled breath condensate IL-8 concentration, dyspnea severity as assessed by MRC scale, lung function measured by spirometry, and functional exercise capacity determined by the 6-MWT.

Data analyses

Analyses were carried out by Statistical Package for Social Science V17.0 [SPSS Inc, Chicago, IL, USA]. Demographic, clinical, and laboratory characteristics at baseline in melatonin and placebo groups were compared by Student's *t*-test for independent samples and by Fisher's exact test, as appropriate. Within-group comparisons (baseline versus after treatment) of Il-8, lung function, MRC scores, and 6-MWT were performed by t-test for paired samples. The time course of the effect of melatonin and placebo on 8-isoprostane level was investigated by repeated measures ANOVA. Additionally, pairwise comparisons (ANOVA) of 8-isoprostane levels at baseline and after the first,

second, and third month of treatment were performed. Differences were considered to be significant at $P < 0.05$.

Results

We studied 36 patients (30 male) with COPD, all ex-smokers, aged 51–80 yr (mean ± S.D. = 66.58 ± 7.81 yr) with BMI ranging from 17.26–34.72 (25.16 ± 4.06) kg/m^2. Melatonin (n = 18) and placebo (n = 18) groups were similar regarding to gender, age, stage of disease, BMI, and smoking history. No significant differences were found at baseline between the two groups in severity of dyspnea, functional exercise capacity, lung function, and concentrations of 8-isoprostane and IL-8 in exhaled breath condensate (Table 1).

Table 1. Demographic, clinical, and laboratory characteristics at baseline of 36 patients with stable chronic obstructive pulmonary disease (COPD), according to treatment with melatonin or placebo

Variables	Melatonin	Placebo	*P* value
Gender, M/F	14/4	16/2	0.65[a]
COPD stage 2/3-4	9/9	8/10	1.00[a]
Age (yr)	68.22 ± 6.87	64.94 ± 8.53	0.21[b]
BMI (kg/m^2)	25.38 ± 4.31	24.93 ± 3.90	0.74[b]
Smoking history (pack-yr)	63.76 ± 39.31	67.83 ± 41.95	0.76[b]
FEV_1	1.42 ± 0.45	1.40 ± 0.43	0.86[b]
FVC	2.79 ± 0.66	2.82 ± 0.67	0.87[b]
MRC scale	2.39 ± 1.46	1.67 ± 1.18	0.11[b]
6-MWT (m)	383.22 ± 40.58	363.48 ± 73.87	0.32[b]
8-Isoprostane	20.40 ± 12.39	16.92 ± 10.33	0.37[b]
IL-8	2.33 ± 0.88	1.98 ± 0.73	0.34[b]

BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MRC, Medical Research Council; 6-MWT, 6-min walk test; IL-8, interleukin 8. Values are mean ± S.D. [a]Fisher's exact test; [b]Student's *t*-test.

A significant decrease in 8-isoprostane levels was observed by longitudinal analysis (repeated measures ANOVA) in patients receiving melatonin, but not placebo (Fig. 2). The 8-isoprostane concentration (mean ± S.E.M.) at each time point was 20.41 ± 2.92 pg/mL (T0), 18.56 ± 2.68 pg/mL (T1), 12.68 ± 2.04 pg/mL (T2), and 12.70 ± 2.18 pg/mL (T3) for the melatonin group and 16.93 ± 2.55 (T0), 16.11 ± 2.54 (T1), 15.72 ± 2.27 (T2), and 17.54 ± 2.22 (T3) for the placebo group. In addition, pairwise comparisons showed a significant difference in 8-isoprostane levels, compared with baseline, after 2 ($P = 0.03$) and 3 months ($P = 0.01$) of treatment, for the melatonin group (Table 2).

Figure 2. Longitudinal analysis (repeated measures ANOVA) of 8-isoprostane levels in exhaled breath condensate of patients with clinically stable chronic obstructive pulmonary disease showing a significant decrease in melatonin (n = 18; solid line) but not in placebo group (n = 18; dashed line), along the 3-month treatment period. Vertical bars represent standard errors.

Table 2. Pairwise comparisons (ANOVA) between 8-isoprostane levels in exhaled breath condensate at baseline and at the end of the first, second, and third study month, in 36 patients with moderate to very severe chronic obstructive pulmonary disease, according to treatment with melatonin or placebo

		Mean difference	S.E.M.	*P* value
Melatonin (n = 18)				
	1st month	1.845	3.181	0.569
Baseline	2nd month	7.729	2.223	0.003
	3rd month	7.708	1.868	0.001
Placebo (n = 18)				
	1st month.	0.816	1.194	0.503
Baseline	2nd month	1.204	1.775	0.507
	3rd month	−0.613	1.333	0.651

Within-group comparisons of IL-8 concentrations in exhaled breath condensate carried out before and after treatment showed a significant increase for the placebo group ($P = 0.03$), which was not observed in patients who used melatonin (Table 3).

Table 3. Interleukin-8 in exhaled breath condensate, lung function, severity of dyspnea, and functional exercise capacity at baseline and after 3 months of treatment with melatonin or placebo, in 36 patients with moderate to very severe Chronic obstructive pulmonary disease

Variable	Melatonin (n = 18)			Placebo (n = 18)		
	Baseline	After treatment	*P* value*	Baseline	After treatment	*P* value*
IL-8 (pg/mL)[a]	2.33 ± 0.88	2.24 ± 0.98	0.60	1.98 ± 0.73	2.41 ± 0.82	0.03
FVC (L)	2.79 ± 0.67	2.76 ± 0.67	0.78	2.82 ± 0.67	2.90 ± 0.70	0.49
FEV_1 (L)	1.43 ± 0.45	1.46 ± 0.54	0.59	1.40 ± 0.44	1.44 ± 0.46	0.55
MRC score	2.39 ± 1.46	1.56 ± 1.38	0.01	1.67 ± 1.18	1.56 ± 1.38	0.59
6-MWT (m)	383 ± 41	391 ± 56	0.39	363 ± 74	389 ± 76	0.06

IL-8, interleukin-8; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; MRC, Medical Research Council; 6-MWT, 6-min walk test. Values are mean ± S.D.

*Student's *t*-test for paired samples; [a]Melatonin group: n = 10; Placebo group: n = 10.

Mean MRC dyspnea score improved significantly after treatment with melatonin ($P = 0.01$), but not with placebo. MRC score at the end of the study was improved in 10 (55.5%) subjects who received melatonin and only in five (27.8%) subjects who took placebo. In the 15 patients with an improvement in dyspnea, we found a significant decrease (baseline *minus* T3) in 8-isoprostane levels (mean difference ±S.E.M. = 5.70 ± 2.21 pg/mL; $P = 0.02$), as opposed to those without improvement (2.0 ± 1.60 pg/mL; $P = 0.22$). No significant changes in lung function (FVC and FEV_1) or functional exercise capacity, as measured by the 6-MWT, were observed in the melatonin or placebo groups (Table 3).

Mild adverse events were reported by two patients from melatonin and two from placebo group, and

included a transient episode of numbness, mild headache, and worsening of dyspnea.

Discussion

These results show that treatment with 3 mg melatonin for two months can reduce oxidative stress, as assessed by 8-isoprostane level in exhaled breath condensate, in clinically stable patients with moderate to very severe COPD. This original finding suggests that exogenous melatonin could have a potentially beneficial effect on disease progression in these individuals.

To our knowledge, this is the first clinical study to look into the effects of melatonin on oxidative stress in COPD. Previously, Nunes et al.[26] showed that melatonin in the same dose as used in the present investigation, taken 1 hr before bedtime for 30 days, can improve sleep in ambulatory patients with moderate to very severe COPD, without any significant adverse impact on daytime sleepiness, lung function, and functional exercise capacity [26] Their report is particularly relevant in view of the high frequency of sleep-related complaints in the COPD population [27, 28], making this substance even more attractive for use in these patients.

Since its antioxidant properties were first described 20 yr ago, melatonin has been recognized by numerous studies as a potent scavenger of reactive oxygen and nitrogen species and also to activate antioxidant enzymes and to up-regulate their gene expression, thereby preventing further oxidative damage [29–34]. The antioxidant capacity of melatonin is even more important owing to its ability to cross all morphophysiological barriers [29]. The subcellular distribution of melatonin allows it to interact with toxic molecules in the entire cell, reducing oxidative damage both in lipid and in aqueous cell environments [15, 29]. In comparison with other antioxidants, melatonin has been found to be more effective than vitamin C in an experimental model of Alzheimer's disease [35] and has shown greater protective effect than vitamin E and N-acetylcysteine against oxidative stress-associated acetaminophen toxicity in mice [36].

The effects of antioxidant treatment have been rarely investigated in patients with COPD, as mentioned earlier. Kasielski and Nowak [9] reported that long-term administration of N-acetylcysteine 600 mg once a day decreased hydrogen peroxide in the exhaled breath condensate of patients with COPD. They studied 44 subjects with stable mild to moderate COPD (22 in the treatment group and 22 in the placebo group), and the decline in hydrogen peroxide levels was not observed before 9 months of treatment [9]. De Benedetto et al. [10] investigated the effect of N-acetylcysteine treatment compared with placebo on oxidative stress in 55 stable patients with moderate COPD and concluded that 600 mg N-acetylcysteine twice a day for just two months could reduce hydrogen peroxide content in exhaled breath condensate [10]. However, a multicentric study of 523 patients with COPD did not show a significant difference between patients using 600 mg N-acetylcysteine daily or placebo in the rate of decline of lung function, exacerbation rate, or health status, during a 3-yr follow-up period [11].

In the present study, oxidative stress was assessed by 8-isoprostane levels in the exhaled breath condensate. In vivo evaluation of oxidative stress can be performed by direct measurement of oxidant production or, indirectly, through quantification of the substances resulting from lipid peroxidation, such as 8-isoprostane, 4-hydroxynonenal and malondialdehyde, in the alveolar space, exhaled air, sputum, or blood [37]. Currently, 8-isoprostane is considered the best available biomarker for oxidative stress because it is relatively stable in isolated samples, it is not influenced by the lipid content of diet, and in vivo formation increases as a function of oxidative stress [38–40]. Collection of exhaled breath condensate is a simple noninvasive method of sampling the lower respiratory tract which has been frequently used for the assessment of oxidative stress in COPD and other lung diseases [4, 41].

Our subjects experienced a significant improvement in dyspnea severity after treatment with

melatonin, without any accompanying changes in lung function or functional exercise capacity. Dyspnea is the major limiting factor for activities of daily living in COPD. The MRC dyspnea scale, among several others, is the most commonly used because of its simplicity, ease of administration, and validation in COPD [24]. It predicts the likelihood of survival [42] and correlates well with health status scores, despite having no significant association with functional impairment in patients with COPD [43]. Previously, a correlation between levels of 8-isoprostane in exhaled breath condensate and severity of dyspnea measured by MRC scale has been described in COPD [44]. In the present study, patients who had an improvement in dyspnea showed a greater reduction in 8-isoprostane levels. There is accumulating evidence that oxidative stress and associated compounds can selectively activate nociceptive airway afferents in bronchopulmonary airways, initiating action potentials in unmyelinated C-fibers that conduct centrally to evoke dyspnea [45], which could at least in part explain our results. It should also be considered that melatonin administration may have led to better scores in the MRC scale through an improvement in sleep and emotional state, which were not directly assessed in the present study. Further investigation is needed to provide a more conclusive explanation for this finding.

An increase in IL-8 level was found at the end of the 3-month study period in patients who used placebo, in contrast to those who received melatonin. IL-8 is considered a key inflammatory mediator in COPD. Increased levels of IL-8, tumor necrosis factor-alpha, and C-reactive protein have been previously correlated with worse disease severity, exacerbation rates, and lung function decline in this disease [46]. Even in mild and moderate exacerbations, increased presence of neutrophils and eosinophils in sputum and airway walls has been associated with higher levels of IL-8 and oxidative stress markers [47]. It has also been recently reported that melatonin can suppress IL-8 production induced by acrolein, a toxic constituent of cigarette smoke, in human pulmonary fibroblasts [48]. Although none of our patients was diagnosed with an exacerbation during the study period, it could be speculated that in subjects receiving melatonin, it may have acted to prevent the rise in IL-8 that was observed in patients who took placebo.

We studied only patients who were in a clinically stable condition. However, it should be appreciated that exacerbations are frequent in COPD and may present with various degrees of severity. A decrease in serum melatonin and antioxidant enzymes activity in erythrocytes together with evidence of increased lipid peroxidation has been previously described during exacerbations in COPD and asthma [49]. Infection is considered the most common cause of exacerbation, and the majority of patients with COPD have been found to carry bacteria in high concentrations in their lower airways during these events [50, 51]. Interestingly, in vitro antibacterial effects of melatonin, particularly against Gram-negative microorganisms, have been reported [52], which could be another potentially useful feature of melatonin for the treatment and prevention of exacerbations in COPD. This issue deserves further investigation.

No significant adverse effects were reported by our subjects. It is generally believed that the acute toxicity of melatonin is extremely low, and systematic review of 17 randomized controlled trials has confirmed that melatonin administration for up to three months is safe. However, there is still a need for studies assessing its safety for more prolonged periods of time [21]. In particular, there has been some concern about the effect of long-term use of melatonin on reproductive function. Previous human studies have produced controversial results, with some showing that exogenous melatonin can affect the spontaneous release of luteinizing hormone and prolactin [53], while others reported that its administration does not influence the secretory patterns of reproductive hormones [54]. In at least one small study, melatonin treatment decreased sperm motility and concentration, which was found in two of eight subjects [55].

In summary, melatonin as used in this study can reduce oxidative stress in patients with stable moderate to very severe COPD, suggesting it could be potentially useful in modifying the progression of this common and frequently lethal condition. Severity of dyspnea may also be

improved by exogenous melatonin, and this could be related to a reduction in lung oxidative stress. These findings, together with previously reported beneficial effects on quality of sleep and in vitro demonstration of antimicrobial activity by melatonin, clearly indicate the need for future long-term studies to assess the effects of this substance on several important outcomes, including lung function decline, exacerbation rates, and health status, in COPD.

Author contributions

AGMC contributed to study design, acquisition of data, data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript, and approval of the article; PFCB contributed to study concept and design, acquisition of data, data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript, and approval of the article; VMSB contributed to data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript, and approval of the article; DMN contributed to acquisition of data, data analysis, and approval of the article; EDBP contributed to study design, acquisition of data, data analysis and interpretation, critical revision of the manuscript, and approval of the article; MMC contributed to acquisition of data, data analysis, and approval of the article; GMA contributed to acquisition of data, data analysis, and approval of the article.

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