



Narcolepsy, autoimmunity, and influenza A H1N1 vaccination

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Narcolepsy is a rare neurological disorder characterized by excessive daytime sleepiness (EDS) with or without cataplexy. A main pathophysiology of narcolepsy is hypocretin deficiency in the central nervous system resulting from a selective loss of hypocretin neurons in the lateral hypothalamus. To date, the pathogenesis of hypocretin neuron loss in narcolepsy is the most commonly accepted autoimmune hypothesis which is supported by genetic risk factors for narcolepsy such as HLA-DQB1*06:02 allele and T-cell receptor alpha polymorphisms. Other evidence supporting the immune-mediated mechanisms include the presence of anti-Tribbles homolog 2 (TRIB2) and anti-streptococcal antibodies in patients with narcolepsy, seasonal patterns of narcolepsy onset, and increased incidence of narcolepsy after the H1N1 pandemic influenza A infections and vaccinations. Among several types of vaccines, the AS03-adjuvanted vaccine Pandemrix (GlaxoSmithKline) was the only vaccine found to increase the risk of narcolepsy. However, the comprehensive results of several epidemiological studies indicate the adjuvant AS03 alone cannot cause the disease. The genetic predisposition, environmental triggers, molecular mimicry of specific H1N1 antigens, and bystander immune activation caused by the adjuvant AS03 may have combined to contribute to autoimmunity against hypocretin neurons and development of narcolepsy.

Keywords: Narcolepsy, Autoimmunity, Influenza A virus, Pandemrix

Introduction

Narcolepsy is a chronic neurological disorder characterized by excessive daytime sleepiness (EDS) with or without cataplexy. Cataplexy occurs in the majority of patients with narcolepsy and refers to a sudden and temporary loss of muscle tone with preserved consciousness [1]. In addition, sleep paralysis and sleep-related hallucinations are included in the classic tetrad of narcolepsy and are implicated with the abnormal regulation of wakefulness and rapid eye movement (REM) sleep [2]. Previously, narcolepsy was classified into two subtypes based on the presence of cataplexy; narcolepsy with cataplexy and narcolepsy without cataplexy. However, hypocretin deficiency has been well established as a key pathophysiology of narcolepsy [3]. Therefore, the classification of narcolepsy in the third edition of the International Classification of Sleep Disorders (ICSD-3) was changed to narcolepsy type (NT) 1 and NT2 depending on

hypocretin deficiency in cerebrospinal fluid (CSF) [4]. This change indicates the diagnostic process has progressed from the clinical phenotype to the molecular biomarker-based approach. The hypocretin deficiency in NT1 is hypothesized to result from the autoimmune process that selectively destructs hypocretin neurons in the lateral hypothalamus. In this review, the clinical features of narcolepsy, autoimmune mechanisms, and issues associated with influenza A H1N1 infection and vaccination are discussed.

Clinical Features

EDS is usually the first and most disabling symptom of narcolepsy. EDS typically presents as constant sleepiness that easily leads to actual sleep episodes or an inability to stay awake. The etymology of narcolepsy is a combination of narke, which means sleep in Greek, and lepsis, which means attack. Accord-

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ingly, EDS can also manifest as sleep attacks and refers to irresistible sleep episodes that may occur while eating, talking, and driving. Sleepiness is relieved by daytime napping; however, the effect is usually temporary in patients with narcolepsy. Although narcoleptics complain of EDS and take frequent naps during daytime, their actual 24-hour total sleep time is comparable with healthy controls. Notably, the quality of nocturnal sleep is worse than in controls. Nocturnal polysomnography (PSG) findings of narcoleptics are characterized by shorter sleep latency, more N1 sleep, less N3 sleep, and more wakefulness after sleep onset, indicating disrupted nocturnal sleep and frequent awakenings [5].

Cataplexy is a more specific symptom of narcolepsy than EDS and is defined as a sudden and temporary episode of muscle weakness triggered by emotional factors such as laughter, anger, and surprise. Cataplexy is present in approximately 60% to 70% of patients with narcolepsy. In mild cases, cataplexy can manifest as jaw sagging, head nodding, and knee buckling. Severe cataplexy can lead to complete patient collapse; however, consciousness is preserved and respiration and eye movements are not affected. Deep tendon reflexes are decreased or absent during the cataplexy attack. Although the exact mechanism of cataplexy remains unclear, an intrusion of the muscle atonia of REM sleep into the waking state is considered a basic mechanism of cataplexy [6]. During wakefulness, hypocretin neurons in the lateral hypothalamus activate gamma-aminobutyric acid-ergic REM sleep-off neurons in the ventrolateral periaqueductal gray, which consequently inhibits the REM sleep-generating neurons in the sublateral dorsal (SLD) nucleus. However, a pathological loss of hypocretin neurons in narcolepsy is hypothesized to lead to disinhibition of SLD neurons and intrusion of REM-sleep muscle atonia during wakefulness. Selective serotonin-norepinephrine reuptake inhibitors and selective serotonin reuptake inhibitors, which are used for pharmacological treatment of cataplexy, inhibit REM sleep generation by activating the monoaminergic neurons [7].

Sleep paralysis and sleep-related hallucinations are other symptoms of the classic tetrad of narcolepsy, although only 15% of patients experience full tetrad symptoms. Sleep paralysis is described as partial or complete muscle paralysis during the onset of sleep or upon awakening in the morning. Hallucinations occur while falling asleep (hypnagogic) or upon awakening (hypnopompic). The hallucinations are manifested as abnormal visual or auditory perceptions which are usually complex, vivid, and dream-like experiences. Sleep paralysis can occur with sleep-related hallucinations. Both sleep paralysis and hallucina-

tions are more frequently found in NT1 than in NT2 [8]. However, these symptoms can also occur in other conditions, such as idiopathic hypersomnia, sleep-related breathing disorders, and normal individuals after sleep deprivation.

Diagnosis

Patients complaining of EDS should be examined for a clinical history of cataplexy, sleep paralysis, and sleep-related hallucinations. A multiple sleep latency test (MSLT) is used to confirm the diagnosis in the evaluation of patients with clinically suspected narcolepsy [9]. Nocturnal PSG should be performed immediately before the MSLT to exclude other significant sleep disorders such as obstructive sleep apnea and periodic limb movement disorder and confirm the patient slept more than 6 hours the previous night. The MSLT consists of five nap trials at 2-hour intervals and the first nap begins 1.5 to 3 hours after the end of the nocturnal PSG recording. The established MSLT criteria for narcolepsy is a combination of a mean sleep latency of fewer than 8 minutes and two or more sleep onset REM periods (SOREMPs). When CSF hypocretin deficiency is confirmed, NT1 can be diagnosed without MSLT results. However, SOREMP (REM sleep latency of fewer than 15 minutes) detected in the overnight PSG can replace one of the SOREMPs on the following MSLT [10]. This is based on previous results showing a SOREMP during the nocturnal PSG is highly specific (>99%) for the diagnosis of narcolepsy with cataplexy, although sensitivity is low 35% to 51% [11]. When there is no clinical history of cataplexy, NT2 can be diagnosed when the MSLT is positive for narcolepsy and CSF hypocretin deficiency is not evident. However, in the diagnosis of NT2 or narcolepsy without cataplexy, the MSLT has poor test-retest reliability [12,13]. Because REM sleep is mainly controlled by the circadian rhythm [14], circadian phase delay resulting from shift work or delayed sleep-wake phase disorder can lead to SOREMPs, especially in the MSLT morning naps. Therefore, prior to diagnosing NT2, potential causes of false positives, such as chronic insufficient sleep, circadian rhythm sleep-wake disorders, shift work, and medications should be excluded by reviewing the detailed clinical history and sleep logs or preferably actigraphy before performing the MSLT [15].

Autoimmune Hypothesis

Hypocretin, also known as orexin, is a neuropeptide produced in the lateral hypothalamus and has a crucial role in the regulation of sleep and wakefulness [2]. Hypocretin contributes to producing and maintaining normal wakefulness by activating

monoaminergic and cholinergic neurons in the brainstem and hypothalamus. The neuropeptide also serves as a buffer between wake-promoting monoaminergic neurons and sleep-promoting neurons of the ventrolateral preoptic nucleus, therefore stabilizing the transition between sleep and wakefulness [16]. A loss of hypocretin neurons in the hypothalamus has been shown to cause narcolepsy. In animal models, hypocretin knockout mice or hypocretin receptor-2-mutated dogs recapitulated major clinical phenotypes of human narcolepsy [17]. In a previous autopsy study, the number of hypothalamic hypocretin neurons in patients with narcolepsy was decreased by 85% to 95% compared with controls. However, significant change was not observed in the number of melanin-concentrating hormone neurons in the hypothalamus between narcoleptics and controls, indicating neuronal loss in narcolepsy occurs selectively in hypocretin-producing neurons [3]. Patients with narcolepsy are not congenitally deficient in hypocretin neurons; the CSF hypocretin level was significantly decreased near the onset of symptoms in a few case studies [18,19].

To date, the autoimmune hypothesis is the most commonly accepted pathogenesis of the loss of hypocretin neurons in narcolepsy. First, a strong genetic association between HLA-DQB1*06:02 allele and NT1 supports the immune-mediated disease mechanism of narcolepsy [20]. HLA-DQB1 encodes the β -chain of major histocompatibility complex (MHC) class II molecules by which antigen-presenting cells present antigens to CD4+ helper T-cells. A genome-wide association study showed that polymorphisms in the T-cell receptor alpha locus were significantly associated with narcolepsy [21]. The results indicated interactions between MHC and T-cell receptors contribute to autoimmunity in narcolepsy. Furthermore, Tribbles homolog 2 (TRIB2), which is normally enriched in hypocretin neurons, was found to be a potential autoimmune target in narcolepsy [22]. Anti-TRIB2 antibody titers were significantly increased within 1 year of narcolepsy onset and were clinically correlated with the severity of EDS and cataplexy. Other evidence supporting the autoimmune hypothesis is a specific seasonal pattern of narcolepsy onset. In a retrospective analysis, narcolepsy incidence peaked in late spring and early summer and increased by approximately six- to seven-fold compared with fall and winter [23]. Winter airway infections likely triggered an autoimmune response to destroy hypocretin neurons with a delay of approximately 6 months until disease onset. Furthermore, the incidence of narcolepsy in 2010 increased three-fold following the 2009 H1N1 influenza pandemic, and in the following year, the narcolepsy incidence returned to baseline, indicating H1N1 infections could be environmental triggers of autoimmune diseases [24]. Streptococcal infections

were also found a possible environmental risk factor for narcolepsy [25]. However, direct evidence does not exist to support the inflammatory reactions in the central nervous system of patients with narcolepsy, which is not consistent with the autoimmune hypothesis [26]. In addition, the disease course of narcolepsy was not altered in most immunomodulating therapy trials [27]. Further studies are needed to clearly understand the autoimmune-mediated disease mechanism of narcolepsy.

H1N1 Pandemic Influenza Vaccine and Narcolepsy

The H1N1 influenza pandemic in 2009 caused 18,500 laboratory-confirmed deaths worldwide and respiratory and cardiovascular deaths associated with the H1N1 pandemic reached 284,400 during the first 12 months of virus circulation [28]. After vaccination against the influenza A (H1N1) pdm09 virus, a rapid increase in incidence of narcolepsy occurred. An annual incidence of narcolepsy in children and adolescents younger than 17 years of age was reported to increase 17-fold and 25-fold after vaccination in Finland and Sweden, respectively [29,30]. In contrast, the association between the influenza H1N1 vaccination and narcolepsy in adults (odds ratio [OR], 1.2; 95% confidence interval [CI], 0.2–9.1) was less than in children (OR, 14.2; 95% CI, 2.5–infinity) [31]. Furthermore, in European countries other than Finland and Sweden, the association was not significant in children (OR, 1.6; 95% CI, 0.5–6.1) and adults (OR, 3.7; 95% CI, 0.7–20.7). Universal immunization for children and adolescents was performed in Finland and Sweden with a high vaccine uptake rate of 50% to 59%, which may explain the regional discrepancy. Conversely, in other European countries, the vaccine uptake rates were low (less than 10%) [31].

Several different vaccines were used against the influenza A (H1N1) pdm09 virus. Adjuvanted vaccines were most widely used in the European countries; approximately 30.5 million people were vaccinated with Pandemrix (AS03-adjuvanted; GlaxoSmithKline, Brentford, UK) and 6.5 million people with Focetria (MF59-adjuvanted; Novartis, Basel, Switzerland) [31]. Notably, analysis of the vaccine brand showed that only Pandemrix had a significant association with narcolepsy. The MF59-adjuvanted pandemic vaccines have not been associated with an increased risk of narcolepsy [32]. Furthermore, in the United States, where only non-adjuvanted monovalent influenza A (H1N1) pdm09 vaccines were used, the incidence rate of narcolepsy did not change before and after vaccination [33]. In South Korea, two different types of vaccines (non-adjuvanted and MF59-adjuvanted) were used, and incidence rates of narco-

lepsy also did not increase in the pandemic or postpandemic periods compared with the prepandemic period [34]. Taken together, the adjuvant, specifically AS03, might have a more important role in the development of autoimmune narcolepsy than the viral vaccine antigen.

Adjuvants are added to a vaccine to potentiate immunogenicity; thus, minimizing the dose of antigen needed. The immunogenicity of the AS03-adjuvanted vaccine, measured based on hemagglutination inhibition titers, was more than 10.5-fold higher than the non-adjuvanted vaccine in children under 3 years of age [35]. Both AS03 and MF59 are squalene-based adjuvants; however, their composition is different. Unlike MF59, AS03 contains α -tocopherol acting as an immunomodulatory component [36], which makes the AS03-adjuvanted vaccine more potent than the MF59-adjuvanted vaccine. However, whether the AS03-adjuvanted vaccine is the sole cause of narcolepsy is questionable. Adjuvants might activate generalized immune responses rather than narcolepsy-specific autoimmunity. However, autoimmune diseases other than narcolepsy did not significantly increase with the use of AS03-adjuvanted vaccine Pandemrix [37]. Although Arepanrix (GlaxoSmithKline) contains the same adjuvant AS03 as Pandemrix, the use of Arepanrix in Canada did not increase the risk of narcolepsy [38]. These findings refute the causal relationship between the adjuvant AS03 and autoimmune narcolepsy. In a mass spectroscopy study on viral proteins, hemagglutinin residue 146N was 10-fold more frequently deamidated to 146D in Arepanrix than in Pandemrix [39]. The subtle differences in viral protein composition of H1N1 vaccines may also have affected susceptibility to narcolepsy development. Furthermore, all children with narcolepsy after H1N1 vaccination were positive for DQB1*0602 or DRB1*15 allele, indicating the genetic predisposition also contributes to the development of vaccine-associated narcolepsy [30].

Summary

Taken together, narcolepsy is considered an autoimmune neurological disease characterized by a loss of hypocretin neurons in the lateral hypothalamus. In the diagnosis of narcolepsy, the MSLT must be carefully interpreted especially in clinical settings where CSF hypocretin measurement is not available or in patients with no clinical history of cataplexy. Significant association between the H1N1 influenza vaccine Pandemrix and narcolepsy has been shown in epidemiological studies. Although the underlying pathophysiology is not fully understood, the multifactorial interactions between the genetic predisposition,

environmental factors, molecular mimicry by specific virus antigens, and immune booster by adjuvants may contribute to the autoimmunity against hypocretin neurons. Further research is needed to develop novel diagnostic and therapeutic strategies targeting the autoimmune pathogenesis of narcolepsy.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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