Inhibition of IgE-mediated allergic reactions by pharmacologically targeting the circadian clock

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Background: The circadian clock temporally gates signaling through the high-affinity IgE receptor (FcεRI) in mast cells, thereby generating a marked day/night variation in allergic reactions. Thus manipulation of the molecular clock in mast cells might have therapeutic potential for IgE-mediated allergic reactions.

Objective: We determined whether pharmacologically resetting the molecular clock in mast cells or basophils to times when FcεRI signaling was reduced (ie, when core circadian protein period 2 [PER2] is upregulated) resulted in suppression of IgE-mediated allergic reactions.

Methods: We examined the effects of PF670462, a selective inhibitor of the key clock component casein kinase 1δ/ε, or glucocorticoid, both of which upregulated PER2 in mast cells, on IgE-mediated allergic reactions both *in vitro* and *in vivo*.

Results: PF670462 or corticosterone (or dexamethasone) suppressed IgE-mediated allergic reactions in mouse bone marrow–derived mast cells or basophils and passive cutaneous anaphylactic reactions in mice in association with increased PER2 levels in mast cells or basophils. PF670462 or dexamethasone also ameliorated allergic symptoms in a mouse model of allergic rhinitis and downregulated allergen-specific basophil reactivity in patients with allergic rhinitis.

Conclusion: Pharmacologically resetting the molecular clock in mast cells or basophils to times when FcεRI signaling is reduced can inhibit IgE-mediated allergic reactions. The results suggest a new strategy for controlling IgE-mediated allergic diseases. Additionally, this study suggests a novel mechanism underlying the antiallergic actions of glucocorticoids that relies on the circadian clock, which might provide a novel insight into the pharmacology of this drug in allergic patients. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Circadian clock, mast cells, basophils, IgE, allergy*

The circadian clock plays a crucial role in the temporal regulation of behavior and physiology, including immunity.[1-7] In mammals the light-entrained central oscillator located in the suprachiasmatic nucleus (SCN) of the hypothalamus synchronizes peripheral oscillators present in nearly all cell types, including mast cells, through neural and endocrine pathways.[1,2] The molecular mechanisms of rhythm generation are highly conserved in the SCN and peripheral cells and created and maintained by interlocked transcriptional-translational feedback loops with a 24-hour period.[1-3] The core feedback loop is driven by 4 clock proteins, 2 activators (circadian locomotor output cycles kaput [CLOCK] and brain and muscle Arnt-like 1 [BMAL1]) and 2 repressors (period [PER] and cryptochrome [CRY]), as well as kinases and phosphatases that regulate the localization and stability of these clock proteins (eg, casein kinase [CK] 1δ/ε). Briefly, CLOCK and BMAL1 heterodimerize and activate transcription of the *Per1* and *Per2* and *Cry1* and *Cry2* genes, as well as other clock-controlled output genes, through E-box or E-box–like elements in the promoter regions of those genes. The PER1/2 and CRY1/2 proteins, in turn, inhibit their own expression by repressing CLOCK/BMAL1 activity. CK1δ and CK1ε play a critical role in the posttranslational modification of the circadian timing system.[8] CK1δ and CK1ε phosphorylate the PER proteins, leading to their ubiquitin-dependent degradation and determine the intrinsic period of the clock. Therefore inhibition of CK1δ/ε slows down PER protein turnover, decelerates clock progression, and lengthens the circadian period.[8]

Recently, the circadian clock has been shown to drive a time of day–dependent variation in IgE/mast cell–mediated allergic reactions in mice.[9-11] The mast cell–intrinsic clock temporally gates FcεRI signaling in mast cells: specifically, FcεRI signaling is reduced at times when the core clock protein PER2 is highly expressed in mast cells (ie, during the active phase in mice).[11] PER2 likely does so in mast cells by inhibiting CLOCK/BMAL1 activity, which regulates expression of the β subunit of FcεRI (FcεRIβ), an amplifier of FcβRI expression and signaling,[12] in a circadian manner through binding to the E-box–like elements in the promoter region of *FCERIB*.[11]

Therefore we hypothesized that pharmacologically resetting the molecular clock in mast cells to times when FcεRI signaling is reduced (ie, when PER2 is upregulated) might inhibit IgE-mediated allergic reactions. To test the hypothesis, this study examined the effects of PF670462, a well-established selective inhibitor of CK1δ/ε, which prevented the degradation of PER2 and upregulated PER2 levels,[8,13-15] or corticosterone, which upregulated *Per2* mRNA and protein levels in mast cells,[11] on IgE-mediated allergic reactions both *in vitro* and *in vivo*.

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Abbreviations used
BMAL1: Brain and muscle Arnt-like 1
BMMC: Bone marrow–derived mast cell
cAMP: Cyclic AMP
CK: Casein kinase
CLOCK: Circadian locomotor output cycles kaput
CRY: Cryptochrome
DEX: Dexamethasone
JCP: Japanese cedar pollen
OVA: Ovalbumin
PCA: Passive cutaneous anaphylaxis
PER: Period
SCN: Suprachiasmatic nucleus
ZT: Zeitgeber time

METHODS

For more information, see the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

PF670462 or corticosterone suppresses IgE-mediated allergic reactions associated with increased PER2 levels in mast cells *in vitro*

We examined the effects of PF670462 or corticosterone on mast cell clockwork (PER2 levels) and IgE-mediated mast cell reactions *in vitro*. Based on monitoring of bioluminescent emission of bone marrow–derived mast cells (BMMCs) from *Per2*^{Luc} knock-in mice, which express PER2 as a luciferase fusion protein (PER2^{LUC} BMMCs),[16] under *in vitro* culture conditions, the time window during which the mast cell clockwork was functional was limited (0-48 hours after a media change for synchronization; Fig 1, *A*).[9,11] Addition of PF670462 (10 μmol/L; a dose that can inhibit both CK1δ and CK1ε activity)[15] or corticosterone (300 nmol/L) 72 hours after the media change (ie, under unsynchronized conditions) significantly increased PER2^{LUC} levels 4 hours later (Fig 1, *A*).

At that time point, PER2^{LUC} BMMCs were sensitized with IgE and stimulated with anti-IgE antibody. IgE-mediated degranulation was inhibited in both PF670462- or corticosterone-treated PER2^{LUC} BMMCs in association with suppression of FcεRI signaling (intracellular Ca^{2+} mobilization and total tyrosine phosphorylation of intracellular proteins; Fig 1, *B-D*). When PER2^{LUC} BMMCs were treated with PF670462 or corticosterone for 15 minutes 72 hours after the media change and then sensitized with IgE, followed by stimulation with anti-IgE antibody, these inhibitory effects were not observed, possibly excluding clock-unrelated or other nonspecific effects (Fig 1, *B-D*). Similar findings were observed in BMMCs from wild-type mice (see Fig E1 in this article's Online Repository at www.jacionline.org). Importantly, the suppressive effects were not observed in PER2^{LUC} BMMCs with a loss-of-function mutation in the key circadian gene *Clock* (*Clock*$^{\Delta 19/\Delta 19}$ PER2^{LUC} BMMCs),[17] in which PF670462 or corticosterone marginally increased PER2 levels (Fig 1, *A-D*). Neither PF670462 nor corticosterone affected cellular viability or baseline cellular bioenergetics of PER2^{LUC} BMMCs (see Figs E2 and E3 in this article's Online Repository at www.jacionline.org). Wild-type BMMCs indeed expressed CK1δ protein (see Fig E4 in this article's Online Repository at www.jacionline.org). Thus resetting (or resynchronizing) the mast cell clock with PF670462 or corticosterone suppressed IgE-mediated reactions associated with increased PER2 levels in mast cells.

We also found that another CK1δ/ε inhibitor, D4476,[18] suppressed IgE-mediated degranulation in wild-type BMMCs associated with increased PER2 levels (see Fig E5 in this article's Online Repository at www.jacionline.org). Furthermore, neither PF670462 nor corticosterone suppressed IgE-mediated degranulation in BMMCs derived from mice with a loss-of-function mutation of *Per2* (*mPer2*$^{m/m}$ mice; see Fig E6, *A*, in this article's Online Repository at www.jacionline.org).[19]

Additionally, we found that a selective inhibitor of CK1ε, PF4800567, which confers greater than 20-fold selective inhibition over CK1δ,[13,20] did not increase PER2 levels and did not affect IgE-mediated degranulation in PER2^{LUC} BMMCs, suggesting that inhibition of CK1δ by PF670462 was more important for this suppressive activity than inhibition of CK1ε (see Fig E7 in this article's Online Repository at www.jacionline.org).

PF670462 or corticosterone suppresses IgE-mediated allergic reactions in IgE-sensitized mast cells, as well as unsensitized mast cells, *in vitro*

We further examined the effects of PF670462 or corticosterone on mast cells that were already sensitized with IgE. The time window during which the mast cell clockwork was functional was not affected regardless of whether mast cells were sensitized or unsensitized with IgE (0-48 hours after a media change for synchronization; see Fig E8, *A*, in this article's Online Repository at www.jacionline.org). Addition of PF670462 or corticosterone, but not PF4800567, 72 hours after the media change (ie, under unsynchronized conditions) significantly increased PER2^{LUC} levels 4 hours later in IgE-sensitized PER2^{LUC} BMMCs, as well as in unsensitized PER2^{LUC} BMMCs (see Fig E8, *A*).

At that time point, the PER2^{LUC} BMMCs were stimulated with anti-IgE antibody. IgE-mediated degranulation was inhibited in both PF670462- or corticosterone-treated, but not PF4800567-treated, IgE-sensitized PER2^{LUC} BMMCs (see Fig E8, *B*). Thus PF670462 or corticosterone suppressed IgE-mediated degranulation in mast cells in association with increased PER2 levels, regardless of whether mast cells were sensitized or unsensitized with IgE.

PF670462 or corticosterone suppresses FcεRI expression in mast cells

To investigate how PF670462 or corticosterone inhibited IgE-mediated degranulation in mast cells, we examined the effects of P670462 or corticosterone on cell-surface FcεRI expression on mast cells. Addition of PF670462 (10 μmol/L) or corticosterone (300 nmol/L) 72 hours after the media change (ie, under unsynchronized conditions) significantly suppressed FcεRI expression in wild-type, but not *Clock*-mutated or *Per2*-mutated, BMMCs 4 hours but not 15 minutes after the treatments (Fig 1, *E*, and see Fig E6, *B*). D4476 also suppressed FcεRI expression in wild-type BMMCs 4 hours but not 15 minutes after the treatment (see Fig E5, *C*). The inhibitory effects of PF670462 or corticosterone on FcεRI expression were also observed when IgE-sensitized BMMCs were treated with PF670462 or corticosterone, but not PF4800567, for 4 hours (see Fig E8, *C*). Furthermore, peritoneal mast cells isolated from wild-type mice 4 hours