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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A1.1

The role of the molecular circadian clock in asthma

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Background: Asthma is a chronic inflammatory lung disease with a strong circadian signature. In 75% of all patients, symptoms worsen overnight; most severe asthma attacks occur at 4 a.m., and the highest number of eosinophils, one of the main effector cells in asthma, is observed in the sputum at this time. Many biological processes such as leukocyte trafficking are controlled by the molecular circadian clock which is driven by interacting feedback loops. As disturbances within the circadian system promotes inflammatory diseases, this project aims to investigate the impact of the molecular circadian clock in asthma. Synthetic compounds such as the RAR-related orphan receptor (ROR) inverse agonist SR1001 target nuclear receptors of the circadian feedback loops pharmacologically, and hence might represent a novel treatment approach in the future.

Methods: Whole blood or isolated immune cells were used for *ex vivo* experiments including chemotaxis, respiratory burst or degranulation assays, or to culture human monocyte-derived macrophages. To investigate the circadian responsiveness, cells were incubated with sera from asthmatic and healthy donors, stimulated with pro- or anti-inflammatory mediators or treated with the ROR inverse agonist SR1001. To study the systematic effect of SR1001 *in vivo*, a murine eosinophil migration model was used. Additionally, the LabMaster system was employed to analyse the effect of pharmacologically targeting the molecular circadian clock on behaviour pattern of the mice.

Results: An oscillating expression of nuclear circadian receptors of the accessory loop in leukocyte subsets including eosinophils, neutrophils, T cells and monocytes was observed by a whole-blood-staining experiment. Comparing expression levels of these circadian receptors from asthmatic and healthy donors, significant differences in distinct components were revealed depending on the time of the day. Similar differences were observed in human polymorphonuclear leukocytes (PMNLs) in a mimicked inflammatory environment using asthmatic sera or pro-inflammatory mediators. The ROR inverse agonist SR1001 reduces the shape, the migratory responsiveness, respiratory burst and the degranulation of human peripheral PMNLs. SR1001 treatment also affects the nuclear circadian receptor expression and polarization of macrophages. Further, systemically applied SR1001 reduces the number of migrated eosinophils in the BAL fluid of IL-5 transgenic mice. Importantly, targeting the accessory loop using SR1001 had no effect on the physiological pattern of activity, drinking or eating rhythm.

Discussion: We observed that the molecular circadian clock oscillates on the receptor level in human leukocytes and is altered under inflammatory conditions such as asthma. Targeting the ROR receptor has an impact on the expression level of the circadian receptors and supresses effector cell functions of eosinophils, neutrophils and macrophages. Further, SR1001 shows anti-inflammatory properties *in vivo,* reducing the migratory responsiveness of eosinophils. Thus, the exogenously applied ROR inverse agonist SR1001 may represent a novel pharmacological approach for the treatment of allergic inflammation and asthma.

Keywords: asthma – molecular circadian clock – inflammation – RAR-related orphan receptor inverse agonists – SR1001

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