CIRCADIAN RHYTHMS AND PERSONALIZED STRATEGIES FOR ANTI-AGING THERAPIES

Olesia Kalmukova, Vitalii Kyryk, Mykola Dzerzhynsky

1Cell and Tissue Culture Laboratory, State Institute of Genetic and Regenerative Medicine, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
2Department of Cytology, Histology and Reproductive Medicine, Educational and Scientific Centre “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine
3D. F. Chebotarev State Institute of Gerontology, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

E-mail: olesiakalmukova28@gmail.com

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Abstract
Background. Nowadays personalized medicine is actively developing and consists of individual approaches during patients’ treatment, diagnoses and prognoses. Since the first use of DNA sequence analysis in 2009, many other directions and methods for precision medicine have been proposed, including metabolome, transcriptome, proteome, microbiome analysis etc., which reflect internal factors of organisms. Moreover, to take into account environmental influence on organisms including day/night activity, feeding and physical training regime, it was proposed to apply the descriptions of circadian system rhythmicity of each patient. Also, with organism aging, the sensitivity to external factors is raised that emphasizes the importance of the chronobiological approach in anti-aging concept. In this review we discussed available ways of the application of circadian system parameters to analyze human metabolic state.

Methods. Search strategy: PubMed, Scopus, DOAJ (Directory of Open Access Journals) and Google Scholar were used to search for original research and articles review; no abstracts from meeting reports have been cited. ClinicalTrials.gov was used to search for clinical studies. Search terms included “chronotherapy”, “circadian system”, and “chronobiology”.

Results. According to personalized medicine, the analysis of circadian system in the case of each patient is necessary as circadian rhythmicity varies in every person. Taking into account the peculiarities of patient’s circadian system it will be easy to choose the best time for drug administration resulting in high efficacy and low side effects. The analysis of circadian system can be performed on molecular, physiological and systemic (general, metabolic and inflammation markers) levels. There was shown the increase in the number of clinical trials which are based on the use of chronobiological approach during the treatment of different pathologies that increase with aging: depression, insomnia, metabolic and cardiovascular disease, cancer. More than 1,000 clinical trials involving circadian interventions and chronobiology have been registered worldwide.

Conclusion. Chronobiological approach can be used as an additional measure to anti-aging therapy to diagnose metabolic state, to choose more effective treatment time as well as in preventive healthcare in terms of personalized medicine.

Keywords: Circadian Rhythm; Chronotherapy; Melatonin; Chronobiology Disorders; Circadian Clocks; Precision Medicine.

Introduction
Circadian system (CS) is the universal mechanism, which provides regulation of change day-night regime in organisms [1]. In a human, circadian timing system consists of hypothalamus suprachiasmatic nuclei (SCN), pineal gland, retina
on the organism or central level; and a molecular clock in each cells of whole organism on molecular or peripheral level organized through neuronal and endocrine signals [2]. On the one hand, changes in normal circadian timing system function (jet-lag [3], shift working [4], artificial light at night [5]) promote desynchrony in an organism, which manifests itself in the stimulated development of different pathologies such as cancer, cardiovascular disease, obesity, diabetes and other metabolic disorders, which only get worse with age [6]. Also, on the other hand, newly arisen disease can influence on circadian rhythm of organisms. Thus, circadian timing system diagnostics can be an important marker of metabolic state, because the dissonance of the peripheral clock leads to circadian rhythm desynchrony of whole body [7].

The main goal of this review was to analyze possible parameters of CS which may be used in personalized medicine for rapid, low-invasive and precise obtaining of the information on metabolic state taking into account the aging changes.

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This narrative mini-review includes:
1. The general organization of CS (systemic, cellular levels);
2. The usage ways of chronobiological approach in personalized medicine;
3. Methods of diagnosing the CS state (systemic, organismal, molecular levels).

Circadian system

Circadian rhythm (“circa” and “dien” – around daily) is cyclic fluctuations in the intensity of biological processes with a periodicity of about 24 hours, which corresponds to the time of rotation of the planet around its axis and is associated with the day and night changing [8]. Circadian rhythms are inherent in all organisms living on the Earth: bacteria, fungi, plants, animals and humans [9].

In mammals, circadian rhythm is regulated by CS, which is organized by central and peripheral parts [10]. Central part consists of master circadian pacemaker – hypothalamus suprachiasmatic nuclei (SCN), which obtain information about outside day/night rhythm via retinohypothalamic tract [11]. Our retina has a special type of intrinsically photosensitive ganglion-cell photoreceptors with photopigment melanopsin that play a crucial role in photoentrainment [12, 13]. Spectral absorption by melanopsin peaks at 479 ± 20 nm, which corresponds blue wavelength [14]. After the activation of melanopsin and intrinsically photosensitive ganglion-cell photoreceptors by blue light in the morning, via retinohypothalamic tract the signal targets SCN, and then the signal transduces in pineal gland across hypothalamus paraventricular nucleus and superior cervical ganglion. In this place, the projection with β-adrenergic mediator is inhibited, thus sympathetic tone of pineal gland reduces. Diminished norepinephrine signaling results in lower concentration of cyclic AMP in pinealocytes, consequently decreases the activity of the melatonin rhythm-generating enzyme in melatonin synthesis reactions – arylalkylamine N-acetyltransferase [15]. As the result, during the day in light-on condition melatonin synthesis is low. At night the activation of melanopsin in intrinsically photosensitive ganglion-cell photoreceptors is absent. Hypothalamus, SCN and paraventricular nucleus don’t get any signals from them. Thus, the sympathetic tone of superior cervical ganglion to pineal gland reappears; norepinephrine signaling induces the rising concentration of cyclic AMP in pinealocytes and the activation of arylalkylamine N-acetyltransferase – melatonin concentration increases [16].

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone of the pineal gland, a derivative of the amino acid tryptophan. Melatonin is a universal molecular marker of changes in time (light) and is synthesized in almost all species of living organisms, including protozoa and plants [17]. In addition, melatonin performs many different functions: from antioxidant in unicellular organisms and integrative in the regulation of mammalian circadian rhythms that are sensitive to photoperiodic changes. At the cellular level, melatonin regulates the mitochondria biogenesis and their activity, antioxidant processes, supports anti-apoptotic signals and is involved in the mechanism of entrainment clock genes of the circadian rhythms [18]. In mammals, melatonin synchronizes the activity of all systems in organism according to the environmental
rhythms; regulates the sleep/wake phase; acts as an immunomodulator; modulates the onset of seasonal reproduction, heat production and changes in energy metabolism through the modulation of hypothalamic signals of eating behavior during the year [19].

Melatonin receptors have been found in all cells of the human body [20]. Melatonin has 3 types of membrane receptors: two of which (MT1, MT2) are associated with G-proteins [21, 22], and one is the cytosolic enzyme quinone reductase MT3/QR2 [23]. Also, melatonin acts on one nuclear receptor (transcription factor) of the retinoic acid superfamily RZR/RORα (retinoid Z receptor α / retinoic acid receptor-related orphan receptor alpha) via sirtuins [24]. Melatonin is also a scavenger of free radicals by binding to them directly [25].

The entrainment of peripheral clock rhythmicity by melatonin in most tissues is organized through the interaction with circadian molecular clock system, which exists in each cells of a human body.

**Circadian molecular clock**

Different organisms use the principle of the transcription/translation feedback loop as a regulatory mechanism of the cell circadian rhythm [26]. The discovery of these universal regulatory mechanisms was marked in science: in 2017 Nobel Prize in Physiology or Medicine was awarded to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for their discovery of molecular mechanisms controlling the circadian rhythm [27].

Circadian molecular clock system has oscillator molecules consisting of positive and negative elements – circadian core clock proteins [28]. In this loop, positive elements activate the expression of clock-controlled genes (CCG). CCG encode, in addition to genes of circadian core clock proteins, genes enzymes, receptors etc. (genes sets differ in various cells). In turn, negative elements inhibit the activity of positive elements. Gradually, the negative elements are phosphorylated and degraded. Free positive elements reactivate the expression of CCG. The feedback loop completes cycle in 24 hours.

In mammals, the positive elements are the heterodimer of Bmal1 (Brain and muscle ARN-t like protein 1) and Clock (Circadian Locomotor Output Cycles Kaput) proteins, and the negative ones are PER (Period)/ CRY (Cryptochrome). In addition, there were identified more circadian core clock proteins: Differentially expressed in chondrocyte 1 (Dec1) and Dec2; REV-ERBα (Nr1d1 – nuclear receptor subfamily 1 group D member 1); Retinoic acid receptor-related orphan receptor α (RORα); casein kinase Iε (CKIε) and casein kinase 1δ (CK1δ) and Timeless (TIM) [29]. Depending on the cell type, genes encoding enzymes, receptors, signals molecules etc. can act as genes whose expression is regulated by a CCG mechanism [30].

This self-oscillating mechanism can be set up and corrected by various factors:

1. Day-night and seasonal changes during year. Melatonin synthesis grows at night time and duration raising its concentration higher at winter night then at summer night [31]. Melatonin targets circadian molecular clock system by REV-ERBα and RORα through indirect modulations or direct binding [32].

2. Physical activity. ATP/ADP ratio (that changing during physical exercise) in mitochondria and its Ca²⁺ mediated signaling influence on the feedback modulation of the circadian clockwork [33, 34]. AMPK or 5’ adenosine monophosphate-activated protein kinase phosphorylates CRY1 and indirectly phosphorylates PER2 by phosphorylating CKIε which leads to the degradation of both CRY1 and PER2 [35].

3. Feeding regime. The resetting of circadian clocks is organized by glucose concentration monitoring (on Dec and Bmal1 genes [35]) and grelin/ leptin ratio. Grelin impacts on the restoration of the circadian rhythm via mTOR/S6 signalling [36, 37].

4. Temperature. Cooling induces nuclear accumulation of transcripts that encode negative regulators (CRY1 and PER2, REV-ERBα) of the circadian clock, which are released into the cytoplasm upon rewarming (Bmal1 inhibited) allowing the synthesis of specific clock proteins [38]. Receptors that transduce signal are TrpM8 and TRPV1 (Transient Receptor Potential Melastatin 8 and Transient Receptor Potential Vanilloid 1) - non-selective cation channels, which target Per1, Per2 and Bmal1 [39, 40].

5. Hormones. As a mediator of feeding behavior, insulin acts on circadian molecular clock via AKT phosphorylation and impact Per1, 2 and
Bmal1 [41]. Glucocorticoid affected on Per1, Cry1, REV-ERBα and RORα [42].

Desynchronization occurs in case of deregulations of peripheral molecular clock with central or between several peripherals molecular clocks. Desynchronization can lead to comorbidity of existing pathologies or provoke the development of new ones [43]. Circadian dysregulations in sleep pattern have consequences in poor sleep, depression, bipolar disorder [44]. Disruptions of circadian system synchronization during aging cause short life span increase the presence of age-related markers [45]. Also disintegration pars of circadian system are displayed in metabolism and can provoke obesity, type 2 diabetes, and fatty liver disease [46].

**Chronobiological approach in personalized medicine**

The application of circadian system analysis in precision medicine is manifested in chronopharmacology and assessment of the body state during pharmacotherapy (for prognosis, diagnosis and treatment). Many proteins have time-dependent expression manner within day because their genes are clock-controlled [47]. A great part of these proteins participate in pharmacokinetics processes – absorption, distribution, metabolism and excretion [48]. For example, peak concentrations of albumin and α-glycoproteins in blood plasma should be taken into account during drug absorption (generally maximum in the afternoon); cytochrome P450, alcohohdehydrogenase, glutathione, methyltransferase, acetyltransferase in the liver – during metabolism of xenobiotics; the presence of membrane transporters in epithelial cells of the intestine and kidneys – during drug distribution and excretion [49].

Top-100 drugs-bestsellers have active components, which interact with proteins that oscillate during day because they are products of clock-controlled genes [50]. For example, esomeprazole that is used in gastritis/esophagitis treatment is interrupted with α subunit of ATPase H+/K+ (ATP4a) [51]; sildenafil that is use for erectile dysfunction treatment connected with cytochrome P450, phosphodiesterase 6G [52, 53]. So, after the analysis of peak expression of target proteins the best administration time of medication is chosen.

According to data from ClinicalTrials.gov website (US National Institutes of Health) at the January of 2022, there are 913 clinical trials involving any category of circadian intervention (for compare at 2016 was registered 348 clinical trials [54]). More than 700 clinical trials are registered in the USA and Europe (Fig. 1). By the topics distribution, a great part of studies is dedicated to diseases of the nervous system (45 %); metabolic disorders (22 %); cardiovascular disease (10 %); cancers (8 %) etc. (Fig. 2). Diseases of the nervous system were proposed to be ameliorated by melatonin usage in different manner and phototherapy (depression, insomnia, migraine, day-night regime disruption).

In case of cardiovascular disease, most of the studies were aimed on the comparison of different time of aspirin or melatonin administration.
Diagnosis of circadian system changes

Before therapy begins at personalized medicine concept, the analysis of CS state is needed for every patient. It is required for more precise diagnosis, treatment and prognosis. In general diagnosis of CS changes can be performed on 3 levels: systemic, physiological and molecular (Fig. 3). At systemic level, several biomarkers can be measured: general, metabolic and inflammation. General parameters include the determination of melatonin and cortisol concentrations during the day by enzyme-linked immunosorbent assay (ELISA), spectrofluorimetry in saliva, urine or blood (sampling at least 4 times a day) [55].

It was shown that the average daily concentration of melatonin and cortisol also decreases in pathologies (obesity, type 2 diabetes, metabolic syndrome, cardiovascular disease, heart attack, pro-inflammatory condition [56, 57, 58]) and with aging [59], not only while seasonal changes. Metabolic markers that have circadian rhythmicity during day involve the measurement of concentration during the day: insulin, leptin, adiponectin and resistin by ELISA in blood (sampling at least 4 times a day). The changes in circadian oscillation of these parameters associate with metabolic syndrome and related comorbidities [60]. Inflammation markers, such as tumor necrosis factor α (TNF α), interleukin-6 (IL-6), interferon γ (INF γ), transforming growth factor β (TGF β), interleukin 10 (IL-10), in blood (sampling at least 4 times a day) indicates the proinflammatory state in the organism [61].

At the physiological level body temperature [62, 63], heart rate [64, 65] and blood pressure [66, 67] can be measured by low-invasive methods using fitness tracker, which constantly obtains information for several days, with further statistical data processing. The connection between changes from normal diurnal oscillation of body temperature, heart rate, blood pressure with hypertension, prediabetes, diabetes type 2, and cardiovascular disease was shown.

At the molecular level, pronounced markers of circadian clock system are the expression of clock genes CLOCK, PER3 after cell culture in vitro and real-time PCR of peripheral blood monocytes or cells from hair follicles (specimen collection at once) [68, 69]. The alteration of clock gene expression is associated with several pathologies, including metabolic syndrome [70].

The changes of CS parameters can also point out the therapy efficiency during aging and diseases in easy way by comparing the dynamics [71].

In the future biosensors which describe CS state quickly and accurately can be developed. For example, in 2020 there was presented a sensor for circadian clock – a passive sweat-based chronobiology tracker that detects circadian relevant biomarkers cortisol and dehydroepiandrosterone (DHEA, poly-functional steroid hormone) [72].

**Summary**

The study of CS parameters is a new direction in personalized medicine. The analysis of these parameters has a practical value, because it can be used as an indicator of therapy efficacy, for metabolic status check and prognosis. For diagnosis circadian system parameters, a wide range of methods can be applied: from molecular to physiological ones. Chronotherapeutic approach does not involve any risk to the patient due to its low-invasive procedure. At present, long-term data including chronopharmacology and age-related comorbidities are need. Future extended researches are required for better understanding of the role of circadian system parameters in the reflection of the organism metabolic state. It is necessary to add the analysis of circadian system parameters in the check-list at anti-aging concept.

**AUTHOR CONTRIBUTIONS**

All authors were involved in the conception of the study. O.K. wrote the first draft of the manuscript based on conversations with all authors. O.K., V.K. and M.D. provided intellectual content, edited the manuscript, approved the final version for
submission and agree to be accountable for all aspects of the study.

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Циркадні ритми та персоналізовані стратегії для анти-вікової терапії

1 Лабораторія клітинних та тканинних культур Державного інституту генетичної та регенеративної медицини НАМН України, Київ, Україна
2 Кафедра цитології, гістології та репродуктивної медицини, Навчально-науковий центр «Інститут біології та медицини», Київський національний університет імені Тараса Шевченка, Київ, Україна
3 Державний інститут героонтології ім. Ф. Чеботарєва НАМН України, Київ, Україна

Резюме

Актуальність. На сьогоднішній день персоналізована медицина активно розвивається і полягає в індивідуальному підході до лікування пацієнтів, діагностики та прогнозування. З моменту першого використання аналізу індивідуальної послідовності ДНК у 2009 році було запропоновано багато інших напрямків і методів для точної медицини, включаючи аналіз метаболіку, транскриптому, протеому, мікробіому тощо, які відображають мультифакторні зміни в організмі. Крім того, для врахування впливу навколишнього середовища на організм, включаючи денну/нічну активність, режим харчування та фізичної підготовки, було запропоновано використовувати оцінку ритмічності циркадної системи кожного пацієнта. Варто зазначити, що зі старінням організму підвищується чутливість до зовнішніх факторів, що підкреслює важливість хронобіологічного підходу в концепції боротьби зі старінням. У цьому огляді ми обговорили доступні способи діагностики параметрів циркадної системи для аналізу метаболічного стану людини.


Результати. З погляду персоналізованої медицини аналіз циркадної системи необхідний у кожного пацієнта, оскільки циркадний ритм є індивідуальним. Враховуючи особливості циркадної системи пацієнта, буде легко вибрати найкращий час для введення певного препарату, що забезпечить його високу ефективність і низький рівень побічних ефектів. Аналіз циркадної системи можна проводити на молекулярному, фізіологічному та системному (загальні, метаболічні маркери та маркери запалення) рівнях. Показано збільшення кількості клінічних досліджень, які базуються на застосуванні хронобіологічного підходу при лікуванні різних патологій, особливо часто збільшується з віком: депресії, безсоння, метаболічних та серцево-судинних захворювань, онкологічних захворювань. У всьому світі зареєстровано понад 1000 клінічних досліджень, що враховують оцінку циркадної ритміки та хронобіологію.

Висновок. Хронобіологічний підхід можна використовувати для діагностики метаболічного стану як доповнення до анти-вікової терапії, для вибору більш ефективного часу лікування, а також для профілактики асоційованої з віком патології з точки зору персоналізованої медицини.

Ключові слова: циркадний ритм; хронотерапія; мелатонін; хронобіологічні розлади; циркадні годинники; точна медицина.