

SYSBIOMED Workshop on Chronobiology, Chronotherapy and SB

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Circadian rhythms determine the sleeping and feeding patterns of most animals, including human beings. The circadian clock, one of evolution's oldest inventions, appeared as early as in cyanobacteria almost 3.5 billion years ago, where it likely is required to synchronise metabolism and sunlight exposure. In multicellular organisms a timing-system orchestrates the activities of large numbers of cells, co-ordinating metabolism and proliferation. There are obvious links of core body temperature, brain wave activity, hormone production, cell regeneration and other biological activities to a daily cycle. The mammalian circadian system is organized in a hierarchical manner in that a central pacemaker in the suprachiasmatic nucleus (SCN) of the brain's hypothalamus synchronises cellular circadian oscillators in most peripheral body cells. Fasting-feeding cycles accompanying rest-activity rhythms are the

major timing cues in the synchronisation of many, if not most, peripheral clocks.

Malfunctioning of the circadian clock is related to transient and chronic disorders of the sleep-wake cycle (jet lag, altered sleep schedule, and delayed sleep-phase syndrome, advanced sleep-phase syndrome, irregular sleep-wake cycle). Surprisingly, there still is no consistent data on the effect of shift work on health.

Chronotherapy

Although sound animal model systems are still missing there is evidence that even very serious diseases - depression, mania and cancers - are connected with dysfunctions of the circadian clock. Whether these correlations represent statistical associations or real causal links still is an open question. Anyway, medical researchers are currently trying to prey on these correlations in chronotherapy by optimising dose-time schedules of medication.

Presumably due to the rhythmic control of hepatic, intestinal, and renal detoxification systems the efficacy of some cytostatic drugs depends on the time of administration. Moreover, the fact that normal cells have their cell cycles coupled to the circadian rhythm could allow for hitting the body system when most normal cells are out of the replicative phase and, therefore, are not that much affected as the de-synchronised dividing cancer cells.

Following this strategy, Francis Lévy presented clinical results of chronotherapy studies in the treatment of colorectal carcinoma (CRC) with oxaliplatin, a cytostatic drug whose clinical development was discontinued by Rhône-Poulenc due to its toxicity. Monitoring the 24h levels of marker drugs (e.g. theophiline) revealed that the amplitudes of drug concentrations in blood can have differences of factor five. Building on this knowledge, Lévy's group succeeded in devising complex delivery schedules, adjusted to the patient's individual rhythms, which allow to apply the drug in a cocktail of cytostatics (5FU-LV-oxaliplatin) allowing for maximum anti-tumour effect while avoiding toxic concentrations. So far, more than 2000 patients have been recruited to chronomodulated therapies. One key finding was that schedules strongly influence the therapeutic outcome. The studies also revealed that there is a clear 'gender effect' in terms of reproducible results which is most likely due to the interfering fertility cycle. It has been known for many years that gene activity patterns of liver cells from male and female mammals display huge differences. Similar differences are observed comparing children and adults. Whether S phase shifts are really relevant for the outcome of the chronotherapy studies was debated in the discussion.

Integrative approaches

The circadian clock is a well-coordinated system, but poorly understood so far. The participants are convinced that a circadian clock and many diseases are mechanistically connected, but consider it a 'hard problem' for research. Disruptions of the circadian rhythm seem to underly phenomena like the higher rate of cardiac stroke patients in the morning half of the day due to the daily blood pressure profile. Investigating internal timing, i.e. individual chronotypes, with regard to the effects of treatment options would be of importance for clinical research, e.g. for studying the effects of pulsed vs. continuous dosing of insulin with diabetics. The striking difference between children and adults in the outcome of leukemia therapies is also suspected to be related to the circadian clock. On the other side, embryogenesis, for example, is obviously de-coupled from the circadian clock. Gastro-intestinal cancers, especially CRC, are considered an expedient model for studying the influence of a circadian clock on tumours, especially valuable when looking for differences between normal and cancer cells. Other types of tumours may also be even more susceptible to biological rhythms. An important aspect of studying the temporal changes in tumours is the formation of metastases which usually tend to degenerate into entities which have lost many traits of the original tumour.

Fundamental questions regarding the relation of the circadian clock, cell cycle, and metabolic rhythm call for intensive research. Fortunately, oscillatory biological processes represent a clear case for systems biology, and modelling is expected to help unravel many of the still unknown mechanisms. For instance, the gating of cell cycle rhythm within the oscillations of the circadian clock is a perfect analogy to a well-known concept from control engineering. Systems biology could also help to analyse data from model systems to discriminate the effects of the circadian clock from genetic factors. Generally, time series experiments must provide quantitative data on multiple parameters ('temporal metabolome') suited to eliminate statistical effects. Different 'chronotypes', i.a. the individual phase relations of organs, are a principal problem of population studies. The integration of models from cells to organs should eventually provide characteristic 'chrono biomarkers' and is also considered essential for optimising personalised chronotherapeutic schedules or finding optimum combinations of drugs. Modeling is, no doubt, well-suited to explain a remarkable result of research in 'advanced sleep-phase syndrome' having found the phosphorylation of a single protein modified by a single mutation responsible for the phase shift.

Data situation

Efforts to build models of the temporal organisation in physiology and disease rely on measuring the absolute changes of parameters. The decay of proteins and changes in various metabolite concentrations seem to be measurable by current methods, while accurate rate coefficients of intracellular reactions are notoriously difficult to measure. Regarding the acquisition of quantitative data the experts suggest to concentrate on preferably non-invasive technologies for multi-parameter monitoring (i.e. simultaneous measurements of skin resistance, temperature, cortisol, FGF, melatonin levels) and on highthroughput data, e.g. from temporal transcriptome profiling (RNAs) employing chip technology. Such efforts could be supported by a ‘chronochip’, or ‘chronosensor’ development. Another concept to obtain temporal personal data is based on reporter cells immobilised in a microreactor which are exposed to a continuous microlitre/minute flow of blood from a cannule. Suited model systems are primary cells, tumour samples and mice *in vivo* models which also would allow for studying the effects of X-chromosome inactivation, gene-knockouts, hormone cycles and gender differences. A lot of high throughput data on liver is already available.

Resources

Clinical researchers are in short supply since most of them are involved in studies within commercial drug approval procedures. Although chronotherapy is a promising field which is closely related to clinical research drawing resources to these projects is considered a hard problem, given present time and funding constraints of academic research. Discussing how to involve researchers from related initiatives and programmes the participants referred to BioSIM, a network of excellence closely cooperating with the pharmaceutical industry aims to develop *in silico* simulation models of cellular, physiological and pharmacological processes. They esteem BioSIM as a valuable measure to generate knowledge by inter-faculty and academia-industry co-operation.

Integrative approaches to chronobiology problems are also comprised by the French ‘Mathematical Analysis of Complex Systems’ initiative, the Swiss ‘SystemsX.CH’ programme and the German ‘Clockwork’ project which is focused on health effects of shift working. Large and smaller industrial entities are known to have launched SB projects. Recently, Pfizer opened a SB division.