Radiation Protection Dosimetry (2016), pp. 1–5 doi:10.1093/rpd/ncw328

THE IMPACT OF CIRCADIAN RHYTHMS ON MEDICAL IMAGING AND RADIOTHERAPY REGIMES FOR THE PAEDIATRIC PATIENT

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Daily rhythmic changes are found in cellular events in cell cycle, DNA repair, apoptosis and angiogenesis in both normal and tumour tissue, as well as in enzymatic activity and drug metabolism. In this paper, we hypothesize that circadian rhythms need to be considered in radiation protection and optimization in personalized medicine, especially for paediatric care. The sensitivity of the eye lens to ionizing radiation makes the case for limiting damage to the lens epithelium by planning medical radio-imaging procedures for the afternoon, rather than the morning. Equally, the tumour and normal tissue response to radiotherapy is also subject to diurnal variation enabling optimization of time of treatment.

INTRODUCTION

Circadian (24-h) rhythms and cell division are fundamental biological systems in most organisms. There is substantial evidence that, in mammals, circadian rhythms affect the timing of cell divisions *in vivo*. This has led to the proposal that circadian rhythms and cell proliferation are phase-locked[1]. Day-night variations in both the mitotic index and DNA synthesis occur in many tissues (e.g. oral mucosa, tongue keratinocytes, intestinal epithelium, skin and bone marrow[2–9]). How the circadian clock controls the timing of cell divisions, however, is not known. Determining how this clock organizes important processes such as cell division, apoptosis and DNA damage repair is key to understanding the links between circadian dysfunction and malignant cell proliferation[10]. It is also central to understanding how best to organize radiotherapy and medical imaging to benefit most the patient, especially for children and younger individuals.

DIURNAL CHANGES IN THE EYE LENS: CELL PROLIFERATION, MELATONIN SYNTHESIS AND REFRACTORY PROPERTIES

Early studies of eye lens proliferation established that there was a diurnal pattern to the proliferation rate in the eye lens[11]. This has been shown for the rabbit[12], rat[13–15], mouse[16] and frog[17]. Interestingly, for some fish the refractive properties of the lens is also diurnally variable[18], a process that is regulated by dopamine[19]. The (rat) lens has capacity to produce melatonin[20] and serotonin N-acetyltransferase levels in the lens can be entrained[21]. So the lens itself potentially contributes to the daily entrainment of the retina[22]. Removal of *Clock1* from the mouse genome correlates with increased age related cataract[23]. The weight of evidence suggests that cell proliferation in the eye lens epithelium is subject to diurnal changes.

Cell proliferation is also age dependent[15]. As proliferating cells are more susceptible to ionizing radiation, young individuals will be more susceptible to damage by ionizing radiation. Preventing cell proliferation protects the lens, but the lens is also the most radiosensitive tissue in the eye[24, 25]. It is for this reason that cataract is one of the iconic non-cancer consequences of IR damage[26]. This coupled to the fact that both oxidative defence mechanisms[27] and the repair of X-ray induced double strand breaks (DSBs) in DNA[28] are synchronized by circadian rhythms also means that the timing of radiotherapy and radio-imaging procedures needs to be coordinated as we enter personalized medicine.

Impact upon the eye lens – the DNA repair response

X-ray exposure induces reactive oxygen species leading to DSBs in DNA[29]. Previous studies determined that the lens epithelium, and specifically the region where epithelial cell proliferation is concentrated, i.e. the germinative zone where IR effects are seen[12, 30] and by preventing cell proliferation, IR-induced cataract is also prevented[31, 32]. Given that the lens epithelium also is subject to circadian rhythm for cell proliferation[11], why is the germinative zone of the lens especially sensitive to IR? DNA repair processes are diurnally regulated in other cells and tissues[28, 33, 34], and DNA repair is regulated

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by melatonin[35], and the lens epithelium has both melatonin receptors and also the ability to synthesize melatonin[20]. We expect DNA repair processes to be diurnally regulated in the lens epithelium, as seen for heart, brain and lung where the levels of DNA repair genes are highest during sleep[36], a time when accumulated DNA damage would be cleared. Therefore timing IR exposures in the late afternoon or early evening would likely help reduce DNA damage and its effects upon the lens epithelium. Moreover, it is clear that reducing proliferation in the lens epithelium is radio-protective[31, 32].

The occurrence and repair of DSBs in DNA differs between the germinative zone and the rest of the eye lens epithelium ([30] and references therein). In this study the mice were all exposed to IR in the morning, the time when proliferation rate would be expected to be highest. Whereas DNA repair in the central, non-proliferative zone was more efficient even than circulating blood lymphocytes from the same animals, lens epithelial cells in the germinative zone had a delayed DNA repair response[30]. Indeed the link between compromised DNA repair and cataract is clear for the lens. For instance in humans, polymorphisms in the *XPD* gene are linked to age related cataract[37] and a recessive *XPD* mutation causes inherited cataract in mice[38]. The levels of acetylated 8-oxoguanine-DNA glycosylase 1 (OGG1), coded by a diurnally regulated gene[33], are elevated in lens epithelial cells of patients with age related cataract[39], with evidence of higher ROS levels. OGG1 is acetylated by sirtuin 1 (SIRT1), a diurnally regulated acetylase[40]. Heterozygosity in ataxia-telangiectasia mutated (ATM) kinase, increased the susceptibility of mice to IR-induced cataract[41], a sensitivity further increased by additional heterozygosity in mRAD9[41], and breast cancer type 1 susceptibility protein (BRCA1)[42] further evidencing a direct link between IR-induced cataract and compromised DNA repair.

Recently the role of Lens derived growth factor (LEDGF/p75) in DNA end resection and homologous recombination has been discovered[43]. LEDGF and RAD51 load onto DNA breaks, but this requires SETD2 (Su(var), Enhancer of zeste, Trithorax-domain containing 2). Loss-of function mutations in SETD2 promotes renal cancers[44]. In the context of this article, LEDGF is required for lens epithelial cell growth and survival[45] and is elevated in response to oxidative stress of lens epithelial cells[46]. Therefore, along with *ATM* heterozygosity, this is another aspect of personalized medicine to be considered in terms of cataract risk.

CHRONOTHERAPY OF CANCER – DIURNAL VARIATION IN THE RESPONSE TO RADIOTHERAPY

Recent work has discovered that the timing of the delivery of ^{131}I in mice influences tissue-specific genome-wide transcriptional responses in a diurnal related manner[47]. The thyroid, liver, and kidney cortex and medulla all showed strong changes in genome-wide expression, and the response in kidney and liver was delayed 3h compared with the thyroid, probably due to indirect effects by thyroid hormone-induced responses that were more evident than the direct response to ionizing radiation[47]. The data suggest that circadian rhythm should be considered not only in radiation research, but also in radionuclide therapy and endpoints considered in the context of diurnal patterns.

Chronotherapy is becoming an emerging aspect of personalized cancer treatment because of the enhancement of both tolerance and efficacy[48–50]. Indeed it is worth considering in this context that the cryptochromes are derived from DNA repair genes[51], and that their removal appears to encourage oncogene transformation[52]. Radiation-induced toxicity is minimized when the timing of radiotherapy treatment is considered. Patients with head and neck cancer, cancer in the lower GI tract and cervical cancer can all benefit from lower side effects when the timing of the radiotherapy treatment is considered (reviewed in[53]). One study on patients with head and neck cancer confirmed morning radiotherapy was better with lower high-grade oral mucositis incidence and reduced weight loss[54], but gender differences were discovered. For women, afternoon treatment regimens were found to be better[54]. Therefore there is capital to be made for patients in terms of reduced side effects from radiotherapy when diurnal considerations are taken into account[55]. It is clear, however, that the interplay between circadian rhythms, cell proliferation and DNA repair with respect to gender and physiological differences need to be better understood (Figure 1).

The cell cycle integrates metabolic, physiological and environmental factors to regulate cell proliferation. DNA repair induced by exposure to ionizing radiation stops the cell cycle, but this effect is dose dependent. Low dose (0.02–0.5 Gy) ionizing radiation can stimulate cell proliferation, while higher doses (>1 Gy) can arrest the cell cycle and then trigger cell death. The cell cycle is diurnally regulated as too is the synthesis of metabolic enzymes and DNA repair proteins. Radiotherapy exploits the fact that dividing cells are more susceptible to DNA damage, but this is not without side effects on normal tissues where cell proliferation is at a much lower rate than in cancers. Understanding the interplay between the cell cycle, circadian rhythm, cell proliferation and DNA repair will deliver benefits to patients by reducing side effects and improving efficacy of radiotherapy treatments and medical imaging procedures.

When discussing optimization of radiotherapy, the influence of circadian rhythm on radiobiological effects on tumour tissue must also be considered in

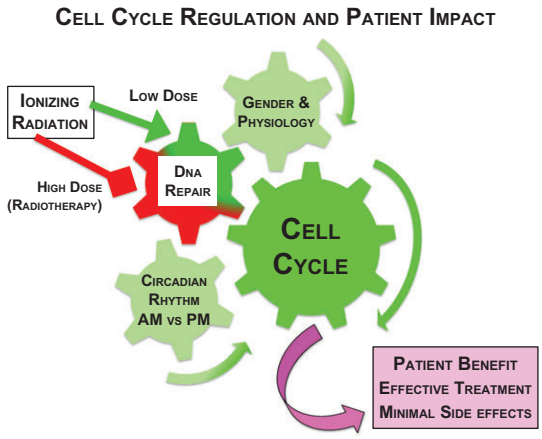

Figure 1. Cell cycle regulation and patient impact.

order to find time points when the therapeutic window is largest. Proliferation of tumour cells seems also to be influenced by rhythmicity. Many tumours follow tumour-specific circadian or ultradian (periods of a few hours) rhythm, different from the normal tissue circadian rhythms[56]. Especially fast-growing or less differentiated tumours seem to follow ultradian pattern. There is thus a potential to find optimal time periods when radiosensitivity is high in tumour tissue while low in normal tissues. It should, however, be noted that radiotherapy may alter the tumour rhythm to become circadian, which must also be considered when optimizing radiotherapy schedule on an individual basis.

There is significant between-subject and circadian variability in enzyme activity and drug metabolism, e.g. for 5-FU[57], which may be used in chemoradiotherapy. In two phase II studies on patients with locally advanced rectal cancer chronomodulated therapy using capecetabine and radiation therapy resulted in effective treatment with low side effects[58, 59].

CONCLUSION

Circadian rhythms serve to synchronize the organism and its physiology to its changing environment and this means integrating, for instance, metabolic processes and the cell cycle as well as coordinating DNA repair. It is therefore not surprising that diurnal patterns significantly impact ageing and cancer susceptibility. Furthermore, radiobiological effects both in normal and tumour tissues are also influenced by circadian rhythms, and some attempts to include such information in clinical studies on radiotherapy and chemoradiotherapy have been performed. Still, more basic knowledge on the relation between radiation and circadian biology is needed, together with preclinical and clinical *in vivo* studies in order to define strategies for optimization of medical use of ionizing radiation.

It is well known that the radiation sensitivity and risk factors are higher for children than adults. One reason is faster cell proliferation leading to higher risk of impairment in DNA repair. Another is the longer life expectancy and therefore the higher risk of long-term consequences of exposure (with longer latency period). Therefore, certainly for paediatric radiotherapy and medical imaging, due consideration needs to be given as personalized medicine becomes the accepted standard.

FUNDING

This work was supported by the University of Durham (32.14.020205) and the Royal Society (IE140736 and IE151271) awards to RAQ, and grants from the Swedish Research Council (grant no. 21073), the Swedish Cancer Society (grant no. 3427), BioCARE – a National Strategic Research Program at the University of Gothenburg, the Swedish Radiation Safety Authority, and the King Gustav V Jubilee Clinic Cancer Research Foundation to EFA.

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