Circadian Cycle and Chronotherapy: Role in the Treatment of Various Biological Disorders

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Abstract: Circadian clocks can be defined as endogenous molecular time-keeping systems that are involved in triggering daily fluctuations in various physiological and biochemical processes. Since early times, the circadian clock has been pragmatic in behavioral and physiological patterns in plants and animals. The circadian clock in humans has a genetic background and many circadian genes have been identified. Important functions of our body such as sleeping, hormone production, digestive secretion and immune activity take place under the influence of the circadian clock in such a way that they occur at times that are most energetically favorable to us. Many studies have suggested that the dysfunction of the circadian system may be associated with an increased incidence of cancer. Cancer chronotherapy is one of the latest developments in the treatment and management of cancer patients. The foundation of cancer chronotherapy lies in utilizing the body’s own circadian (Biological) clock to help the patient maximise the efficacy of their treatment. This brief review substantiates the important role played by circadian clocks in the development and the therapeutic efficacy of anticancer agents.

Key words: Circadian Clocks • Cancer • Chronotherapy • Rhythm • Drug Delivery

INTRODUCTION

Since from early times, daily rhythm in plants and animals have been observed. In 4th century BC, Alexander the Great’s Scribe Androstenes observed that leaves of some trees opened during day and closed during night, that shows clear phenomenon of rhythmicity [1, 2]. Behaviors of circadian rhythm in mammals are well known robust and precise. Drug efficacy and toxicity depend upon the relationship between dosing time and 24 hours rhythm of biochemical, physiological and behavioral processes. Many drug cause alteration in 24 hours circadian rhythm which leads to homeostatic irregulation and adverse effect, which can be minimized by reducing dosing schedule [3]. The word circadian is derived from the Latin circa which means “about” and dies which can be defined as “a day”. Normally, circadian rhythms are self-sustaining, endogenous oscillations that are synchronized according to internal biologic clocks related to the sleep-wake cycle. Table 1. Shows the various measurements of biological rhythm [4]. Several functions of the human body vary day by day of a disease over a certain period of time because these types of variations cause the changes in disease state and in plasma drug concentrations. The pain is increases when sleep disturbances are occur due to the hormonal levels are at peak. Human circadian based on sleep-activity cycle or solar / lunar which is influenced by our genetic makeup and affects the body’s functions day and night (24-hour period) [5, 6]. Circadian rhythm regulates many functions in humans’ body like production of hormones, sleeping pattern, behavior and physiology [7, 4]. The main aim of Chronotherapeutic drug delivery systems (CDDS) is to meet the therapeutic needs for treatment according to the pathological diseases chronotherapeutics drug delivery offers a new condition [8]. It refers to a treatment method in which in vivo drug availability is timed to match rhythms of disease in order to optimize therapeutic
Main rhythmic components

| Period (τ) | Short Period  
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<td>τ&lt;0.5 h</td>
<td>s&lt;τ&lt;1 s</td>
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<td></td>
<td>Pulsatiles</td>
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<td></td>
<td>τ– min</td>
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<td>Intermediate Period</td>
<td>Circadian (20 h&lt; τ&lt;28 h)</td>
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<tr>
<td>[0.5 h&lt;τ&lt;6 days]</td>
<td>Ultradian (0.5 h&lt;τ&lt;20 h)</td>
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<td>Infradian (28 h&lt;τ&lt;6 days)</td>
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<td>Long Period</td>
<td>Circamensual (τ~30 days)</td>
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<td>[τ~6 days]</td>
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<td>Circannual (τ~1 year)</td>
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Table 1: Shows the various measurements of biological circadian rhythm

outcomes and minimize side effects. It is depend upon the observation that there is an interdependent relationship between the peak-to-trough rhythmic activity in disease symptoms and risk factors, pharmacologic sensitivity and pharmacokinetics of many drugs [9]. Chronopharmacotherapy means that drug regimen depend upon circadian rhythm, is recently gaining much attention throughout the worldwide. Most of the diseases like arthritis, acidity asthma and hypertension show variation in circadian rhythms, which required drug release at scheduled-time for effective action, e.g. inflammation associated with morning stiffness, asthma and heart attack in the early hours of the day. To follow this principle, one must have to design the dosage form such that it can be given at the convenient time, e.g. bed time for the above-mentioned diseases with the drug release in mornings compared with evenings and site-specific absorption from the small intestine. Drug pharmacokinetics show circadian variation for various anti-inflammatory drugs like aceclofenac diclofenac sodium, indomethacin and ketoprofen which have a greater absorption in the morning as compared with the evening and site-specific absorption from the small intestine. Therefore, to develop dosage forms for chronopharmacotherapy, the designed drug release should be time-specific as well as site-specific [10]. There are number of hormones such as progesterone and estrogen and are released by the brain in the morning, while others are released during sleep such as melatonin and cortisol. Blood pressure and heart rate are highest during the hours of 6.00 a.m. to 12.00 noon [8, 11]. To determine the timing and amount of medication chronotherapy considers a person’s biological rhythms to obtain a maximum drug’s desired effects and to minimize the undesired. Due to understanding of biological time, the idea came that these biological rhythms must affect how the body responds to drugs administered over the course of the day [12, 13]. The chronotherapeutics approach is based on the resynchronizing the circadian rhythms that control, synthesize and release the important catecholamine and indoleamine neurohormone. Mostly the heart attack stokes occur 7am and noon. This is elevated with increases in the pulse rate, blood pressure and platelet aggregability while plasma fibrinolytic ability is low during the day [14].

**Circadian Time Structure**: The human circadian time structure is always peak for 24 hr. The peak time of human circadian rhythm is in the synchronization with the routine sleep in darkness from 10: 30 pm to 6:30 am and activities during the light of the day between 6: 30 am and 10:30pm. These rhythms are helpful in defining the temporal organization of the human beings. The human circadian time structure is to depict the peak time of 24-h rhythms on a clock basal gastric acid secretion, white blood cell count (WBC), calcitonin a gene-related protein and arterial natriuretic peptide occurs late at night or early in sleep. Growth and thyroid stimulating hormone (TSH), blood lymphocyte and eosinophil number and plasma melatonin and prolactin crest during sleep as the adrenocorticotropic (ACTH), follicle stimulating (FSH) and luteinizing (LH) hormones and plasma cortisol, rennin activity, angiotensin and aldosterone are peak in the morning. The circadian rhythms of serum cholesterol and triglycerides and urinary diuresis crest early in the evening.

The circadian clock can act as an instrument for the estimation of the day and night length and for seasonal phenomena. It also acts as a clock for changing the day and night length which can be regulated appropriately [15]. The pair of supra chiasmatic nuclei are situated in the hypothalamus. The pineal gland controls the circadian rhythms that control, synthesize and release the important catecholamine and indoleamine neurohormone. Mostly the heart attack stokes occur 7am and noon. This is elevated with increases in the pulse rate, blood pressure and platelet aggregability while plasma fibrinolytic ability is low during the day [14].

Chronotherapeutic systems have been designed due to certain reasons as cited below:

- **Circadian rhythms play an important role in the pathophysiology of diseases** (Chronopharmacotherapy).
- To prevent degradation of drugs such as proteins and peptides in upper gastrointestinal tract.
- Delivery of hormones in a programmed manner, as continuous release dosage forms may lead to disturbance in normal feedback mechanism as well as resistance may also take place.
Advantages of Chronotherapy [18, 12]:

- This therapy is drug free.
- It is more effective when a person falls asleep for long or several hours.
- Improves patient confidence and condition while chronotherapy patient often falls asleep.
- Prediction can be done easily of the point at which it will work because chronotherapy is different from other treatments as its got the beginning, middle and an end.
- There is no risk of dose dumping.
- Stability improved.
- It gives a new schedule like getting up and sleeping early which will be quite unusual for some days but it will give period to adjust psychologically.

Disadvantages of Chronotherapy [19, 12]:

- It develops a non 24 hours sleep wake syndrome after the treatment as the person sleeps for or over 24 hours during the treatment.
- Person become less productive during chronotherapy and staying awake till the other schedule will be bit uncomfortable.
- Medical supervision is mandatory for this therapy.
- Large number of process variables.
- Trained /skilled person is needed for manufacturing.
- Persons going through this therapy unusually may feel hot and cold sometime.
- Regular consultation from sleep specialist is recommended.
- Patients have to consult with doctor at regular time period to avoid side effects.

Chronopharmaceutics: The term Chronopharmaceutics is composed of two words “chronobiology” and “pharmaceutics”. Chronobiology is the study of biological rhythm and their mechanism, whereas the study of influence of biological rhythm on the effects of medication is known as chronopharmacology. Chronotherapeutics is based on the biological clock which is responsible for controlling many vital activities of the physiological organs of the human body. Circadian rhythms are the main rhythm in the chronotherapeutics and the dysfunction of circadian rhythms may affect the functioning of the brain and it can be improved by the chronotherapeutics approach.

Chronotherapeutics is the clinical practice of harmonizing drug delivery in accordance with body's circadian rhythm including ailment states for the purpose of maximising benefits and minimizing the harm [21]. Biological rhythms at the cellular and sub cellular level can give rise to significant dosing-time differences in the pharmacodynamics of medications that are unrelated to their pharmacokinetics. This phenomenon is termed chronesthesia. There are a number of conditions that show a circadian pattern and adjusting the administration of drugs according to the circadian rhythm of the disease state can significantly benefit such diseases, such as:

- Hypertension
- Myocardial infarction
- Bronchial asthma
- Peptic ulcer
- Arthritis
- Hypercholesterolemia
- Allergic rhinitis
- Alzheimer’s disease
- Sleep disorders
- Cardiovascular diseases

Drugs that are formulated for chronotherapy are regulated by the Food and Drug Administration.

Diseases Affected by the Biological Rhythm:

Chronic therapy for Asthma: It has been estimated that occurrence of symptoms of asthma is 50 to 100 times more often at night than during the day [23]. Airway resistance,
bronconstriction and exacerbation of symptoms increase progressively at night in asthmatic patients. The various circadian-dependent factors that contribute to the worsening of nocturnal asthmatic symptoms are cortisol (an anti-inflammatory substance) levels are found to be highest at the time of awakening and lowest in the middle of the night and histamine (a mediator of bronchoconstriction) concentrations are peak at 4:00 am [24]. Daily or alternate day, morning dose of glucocorticoid medications such as methyl predisolone (Medrol) significantly moderates side effects and enhance therapeutic benefits. Oral predisolone administered at 3 pm rather than at 8 am has been shown to be highly effective in the treatment of nocturnal asthma. Evening once daily dosing of controlled release theophylline tablets (Uniphyl 400 mg tablets) showed chronotherapeutical potential in the treatment of nocturnal and early morning asthma [25].

Chronotherapy for Cardiovascular Diseases: The differences in patterns of illness between day and night for cardiovascular disorders such as hypertension, angina, heart attack, sudden cardiac death and stroke have been documented. The blood pressure of hypertensive patient increases rapidly in the morning after awakening, peaks in the middle to late time of the day, decreases in the evening and is lowest while the patient is asleep at night. The risk of heart attack is also greatest during the early morning hours after awakening. This may be attributed to various factors such as capillary resistance and vascular reactivity is higher in the morning and decrease later in the day. Platelet aggregability is increased and fibrinolytic activity is decreased in the morning, leading to a state of relative hyper coagulability of the blood.

Chronotherapy for Arthritis: Patients with osteoarthritis tend to have less pain in the morning and more at night; while those with rheumatoid arthritis, have pain that usually peaks in the morning and decreases throughout the day. For osteoarthritis sufferers, the optimal time for a nonsteroidal anti-inflammatory drug such as Ibuprofen is therefore around noon or midafternoon. The same drug would be more effective for people with rheumatoid arthritis when taken during evening. There is a circadian rhythm in the plasma concentration of C - reactive protein and interleukin-6 of patients with rheumatoid arthritis [26]. The new cyclooxygenase-2 inhibitors are helpful in relieving the osteoarthritis symptoms when taken in the morning and better results are obtained in rheumatoid arthritis when the dose is taken in the evening [27].

Chronotherapy for Sleep Disorder: The time of sleep required by each person is usually constant, although there is a wide variation among individuals. Any disturbance in the circadian rhythm, or any abnormality in the individual processes during sleep, may result in a variety of disorders such as delayed sleep phase syndrome which is characterized by severe sleep-onset insomnia. Normally, sleep is impossible until 3 a.m. or later until there is great difficulty in awakening in the mornings at the normal time. The ability to cope up with circadian rhythm disturbances also differs from person to person. Identification of the individual variation would be of importance in dealing with certain sleep disorders [28].

Classification of Sleep Disorders [29]

Dyssomnias:
- Intrinsic sleep disorders
- Extrinsic sleep disorders
- Circadian rhythm sleep disorders

Parasomnias:
- Arousal disorder
- Sleep – wake transition disorders
- Parasomnias associated with REM
- Other parasomnias

Sleep Disorders Associated with Medical / Psychiatric Disorders:
- Associated with mental disorders
- Associated with neurological disorders
- Associated with other medical disorders

Chronotherapy for Allergic Rhinitis: Early-morning sneezing, nasal congestion and runny nose are common in allergic rhinitis. Study also explained that a morning dose of antihistamine was not as successful as the same dose given in the evening [30].

Chronotherapy for Peptic Ulcer: The maximal acid secretion, pain associated with peptic ulcer as well as perforation of gastric and duodenal ulcer are more frequent at night time. The nocturnal administration of drugs results in more effect control of the disease [31]. Nocturnal administration of the peptic ulcer medicines not only reduces the acid secretion more effectively but also promotes the ulcer healing and reduces ulcer recurrence [32].
Chronotherapy for Hypercholesterolemia: Discovery of the circadian rhythm of cholesterol biosynthesis gave a new turning point [33], where it was stated that elevated rates of cholesterol intake and hepatic cholesterogenesis takes place during the evening hours irrespective of fed/fasting state. An evening administration of an HMG-CoA reductase inhibitor lowered serum cholesterol levels than morning dosing.

Chronotherapy for Diabetes: The most widespread application of chronotherapy is the insulin pump, which is used to administer insulin for the treatment of diabetes mellitus. With the insulin pump, patients can customize insulin delivery to meet their particular requirements. Several systems that respond to changes in glucose concentration have been developed like pH sensitive hydrogel containing glucose-oxidase enzyme immobilized in hydrogel. As the blood concentration of glucose rises, glucose-oxidase converts glucose into gluconic acid, which changes the pH of system. Due to change in pH, swelling of polymer takes place and this result into insulin release. Insulin decreases the blood glucose level and consequently the gluconic acid level also declines and system turns to deswelling and hence decreasing the insulin release [34].

Chronotherapy for Pain: Circadian rhythms in acute pain have been documented, such as in dental surgery, with a morning peak during the first postoperative day. The peak of morphine use occurred at 09:00 h and was the least at 15:00 h in patients undergoing elective surgery. The peak demand for morphine or hydromorphone occurred in the early morning and was lowest during the night in postoperative gynecologic patients [35].

Chronotherapy for Epileptic Seizures: Chronobiology comprises some working hypotheses in phychophysiology and permits the development of new theoretical concepts in the field of neurological sciences. It is also well known that the brain area with the highest concentration in noradrenergic nerve terminals and nonadrenergic nerve terminals and noradrenaline (NA) have a circadian rhythm in their content of NA [36].

Chronotherapy for Myocardial Infarction: Onset of myocardial infarction has been shown to be more frequent in the morning with 34% events occurring between 6 am and noon. Acute cardiac arrest and transient myocardial ischemia shows an increased frequency in morning, the causes being the release of catecholamines, cortisol, increase in the platelet aggregation and vascular tone [37].

Development of Chronotherapeutic Delivery Systems: Several techniques for desired drug release have been developed and applied to design chronopharmaceutical delivery systems. Different chronopharmaceutical technologies and marketed products are given in Table 2. These techniques are broadly classified into following three major categories:

- Time controlled chronotropic systems.
- Stimuli induced pulsatile drug delivery systems
- Externally regulated pulsatile drug delivery systems

A basic chronotherapeutic system consists of a drug containing core and a barrier layer of polymer to control drug release from the core.

Time Controlled Chronotropic Systems: The drug is released as a burst within a short period of time immediately after a pre-determined off release period.

Time Controlled Chronotropic Systems Based on Capsules: These systems are composed of an insoluble capsule body, swellable and degradable plugs made of hydrophilic polymers (Like hydroxyl propyl cellulose, poly vinyl acetate, polyethylene-oxide), lipids and bioactive molecule. The lag time is controlled by plug, which is pushed away by swelling or erosion and drug is released as a pulse from the insoluble capsule i.e. Pulsincap® a swellable hydrogel seals the drug contents into the capsule body. The hydrogel plug swells when the capsule comes in contact of fluid and after a lag time, the plug pushes itself outside the capsule and rapidly releases the drug [38].

Time Controlled Reservoir Systems with Rupturable Polymer Coating: The core is coated with a protective polymeric rupturable layer and an outer water insoluble semi permeable rate controlling membrane. Pressure is required to rupture the coating which can be achieved by using swelling agents, gas producing effervescence agents or osmogens [39-42]. Swelling agent includes superdisintegrants like carboxy methylcellulose, sodium starch glycinate, L-hydroxy propyl cellulose. Polymers like polyacrylic acid, polyethylene glycol etc and a mixture of tartaric acid and sodium bicarbonate is used as effervescence agent. Water ingress to system causes the coating to swell, rupture and release of drug occurs. Release of drug is independent of pH or solubility of drug. Lag-time can be varied by varying thickness of coating or by changing amount of plasticizers in the outermost
Table 2: Various developed chronopharmaceutic systems [48]

<table>
<thead>
<tr>
<th>Active pharmaceutical Ingredient (api)</th>
<th>Proprietary name</th>
<th>Proprietary chronopharmaceutical technology</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem HCl</td>
<td>Cardizem® LA</td>
<td>CEFOR® technology</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Propranolol HCl</td>
<td>InnoPran® XL</td>
<td>DIFFUCAPS® technology</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Verapamil HCl</td>
<td>Covera-HS®</td>
<td>OROS® technology</td>
<td>Hypertension</td>
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<tr>
<td></td>
<td>InnoPran® XL</td>
<td>DIFFUCAPS® technology</td>
<td>Hypertension</td>
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<td></td>
<td>Verelan® PM</td>
<td>CODAS® technology</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Famotidine</td>
<td>Pepcid®</td>
<td>Physico-chemical modification of the API</td>
<td>Ulcer</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Lipovas®</td>
<td>Physico-chemical modification of the API</td>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Uniphyl®</td>
<td>CONTIN® technology</td>
<td>Asthma</td>
</tr>
</tbody>
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layer. Rapid release of drug after lag-time can be observed with increase in the concentration of the osmotic agent [43-45].

Time Controlled Reservoir Systems with Soluble or Eroding Polymer Coating: Ethylcellulose (EC) of varying particle sizes has been used as an outer coating layer to design a novel dry-coated tablet of sodium diclofenac by direct compression for time-controlled drug release. The drug release from dry-coated tablet exhibited an initial lag period depending on the particle size of the EC powder, followed by rapid drug release. The smaller the EC particle size used the longer the lag time obtained, which suggests that the particle size of EC powder could modulate the timing of drug release from such a dry-coated tablet. The period of the lag time for sodium diclofenac released from dry-coated tablets was correlated with the penetration distance of the solvent into dry-coated tablet by an in vitro dye penetration study. The results suggest that these dry-coated tablets prepared with different particle sizes of EC powder as an outer coating layer might offer a desirable release profile for drug delivery at the predetermined time and sites [46].

Pulsatile Systems Based on Changed Membrane Permeability: Change in permeability of polymeric coating layer is responsible for drug release in presence of certain counter ions of surrounding media [47].

Stimuli Induced Pulsatile Drug Delivery System: The drug release from these systems is based on the physiochemical processes of body. These systems are meant for site specific targeted drug delivery by the induction of various physiochemical stimuli at target site. Biological stimuli like release of enzymes, hormones, antibodies, pH of the target site, temperature of the site, concentration of biomolecules (Glucose, neurotransmitters, inflammatory mediators) etc acts as stimuli to trigger the release of drug from these types of drug delivery systems. These systems are classified into following sub categories:

- Chemical stimuli induced pulsatile drug delivery systems
- pH sensitive pulsatile release chronotropic systems
- Enzyme catalyzed pulsatile chronotropic systems
- Temperature induced pulsatile drug delivery systems

Externally Regulated Pulsatile Drug Delivery Systems: External stimuli like ultrasound, magnetic field, electrical effect and irradiation are required to control the drug release from these types of systems. When these external factors are applied on the delivery system, conductors present in the delivery system get sensitized and trigger the release of drug from the delivery system. Magnetic beads prepared by interfacial polymerization of polyamide microcapsules shows this type of delivery mechanism.

Various technologies to develop time controlled peroral drug delivery systems have been extensively studied in recent decades. Some of these systems are discussed as follows:

Enteric-coated Systems: Enteric coatings have been used to prevent the release of a drug in the stomach (Fig 2). Enteric coatings are pH sensitive and drug is released when pH is raised above 5 in the intestinal fluid. These formulations can be utilized in time-controlled drug administration when a lag time is needed. Such systems cannot be the first choice when a time-controlled release is required because of the unpredictability of gastric residence. In the treatment of nocturnal asthma, a salbutamol formulation containing a barrier coating which is dissolved in intestinal pH level above about 6, has been successfully used [49]. The system contains a core which is film coated with two polymers, first with HPMC and then with a gastro-resistant polymer
polymer to achieve different lag-times [53-55]. By applying different coating thicknesses, lag times in vivo of up to 5 hours can be achieved. Release rates from SRS, beyond the lag time, has been found to be independent of coating thickness.

Press-Coated Systems: Delayed-release and intermittent-release formulations can be achieved by press-coating. Press-coating, also known as compression coating, is relatively simple and cheap and may involve direct compression of both the core and the coat, obviating the need for a separate coating process and the use of coating solutions. Materials such as hydrophilic cellulose derivatives can be used and compression is easy on a laboratory scale. On the other hand, for large-scale manufacture, special equipment is needed. The major drawbacks of the technique are that relatively large amounts of coating materials are needed and it is difficult to position the cores correctly for the coating process [56].

Pulsinocap: It is a delivery system which releases drug contents at a predetermined time or at a specific site within the gastrointestinal. Each capsule is composed of a water insoluble body and a water soluble cap and also contains the drug dose which is sealed with a hydrogel plug. At a predetermined time after ingestion, the swollen plug is ejected from the capsule and the drug is then released into the small intestine or colon. The dimension of the plug and its position in the capsule can be varied and the system delivers drug at exactly the programmed time, 1 to 10 hours after drug administration, to various regions of the gut [57].

The relevance of Chronotherapeutics in Cancer Therapy: One of the first diseases studied that showed progressed clinical outcomes with chronotherapy was acute lymphoblastic leukemia [58]. Experimental studies in cancer chronotherapy were initially performed by Halberg et al. [59]. Hrushesky et al. [62] conducted research on chronotherapy for gynecological and genitourinary cancers including advanced renal cell carcinoma. These studies demonstrated the superiority of chronotherapy with respect to response and side effects when compared to conventional chemotherapy [60, 61]. Phase I-III clinical trials showed chronotherapy significantly increases tolerance to high doses of anti-cancer drugs and improves antitumor activity in patients with metastatic colorectal cancer [62].
Cancer chronotherapy is one of the latest developments in the treatment and management of cancer patients. Cancer chronotherapy involves using the body’s own circadian (biological) clock to help the patient in maximising the efficacy of their treatment.

There are 3 distinct components in the circadian clock in humans. These include the input pathways, the central pacemaker and the output pathways. The function of the input pathways is to relay sensory information to the central pacemaker concerning external cyclic cues (e.g. light and feeding times). The output pathways perform the function of physically synchronizing the behaviour and physiology with the circadian rhythm. The central pacemaker on the other hand relays the signal messages between the output and input pathways in a rhythmic fashion. The central pacemaker in mammals is in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus [63] which plays a key role in the coordination of circadian rhythms [64].

Light is considered as the most important of all the environmental stimuli. However, the pathways that detect light for the purpose of circadian synchronisation are different from those that are used for visual perception. This is why certain blind human subjects still retain their circadian rhythm despite having no perception or awareness of light [65].

The suprachiasmatic nuclei (SCN) make it possible to predict the rhythmic aspects of cellular metabolism and proliferation. Synchronized individuals display circadian rhythms with predictable times of peak and trough. These rhythms may influence the pharmacology and the tolerability of anticancer drugs and/or their antitumor efficacy. Conversely, a lack of synchronization, or an alteration of circadian clock function makes rhythm peaks and troughs unpredictable and may require specific therapeutic measures to restore normal circadian function.

A cancer can be defined as an uncontrolled proliferation of cells because of irregularities in the regulation of the cell cycle. This may be fast or slow but the cells never stop dividing. In order to achieve cancerous growths, several mutations must take place to prevent the natural defence of the cell cycle against cancer. The development of a cancer takes place once sufficient damage to DNA is caused. A single mutation may express itself and give the cell a slight growth advantage over the surrounding cells.

The circadian clock can be called as tumour suppressor in some respects as it affects the body at a systemic and cellular level in order to control the proliferation of cells. At the cellular level, the circadian clock controls the cell cycle progression by regulating the expression of clock-controlled genes. Several studies show a strong correlation between the rhythmicities of the expression of circadian genes and various cell cycle phases. Therefore, the circadian clock can indirectly control the expression of genes that regulate the ‘checkpoints’ between the different phases of the cell cycle. Thus, the central pacemaker can affect the expression on tumour-suppressing genes and regulatory proteins at the cellular level.

The central pacemaker also performs the function of regulating the release of endocrinal hormones and has a lot of influence on the neuroendocrinial systems. The neuroendocrinial systems secrete hormones such as oestrogen and glucocorticoids to affect their endocrinal targets. These hormones are under the influence of the circadian clock as 24 hour rhythmic changes in their levels in the body can be monitored. Therefore, any interruption of the circadian clock will ultimately have an adverse affect on the secretion of these hormones and as a result disrupt the rate of cell proliferation.

The neuroendocrinial systems also play an important part in the immune response as many of the hormones control cytokine production and leukocyte production and distribution. This leads to the conclusion that the immune response is also under the influence of the circadian clock and hence giving rise to another method of tumour suppression i.e. immunomodulation. As a result, a disrupted circadian clock can lead to a compromised immune system which in turn leads to decreased immunosurveillance and cancer development.

The Rest-Activity Rhythm: A Relevant Marker of the Circadian Clock: Locomotor activity is a reflection of circadian clock function in several animal species. Its endogenicity was demonstrated by its persistence in constant environmental conditions in flies, rodents and humans. This rhythm is controlled by the molecular clock genes in mammals. Direct pharmacologic actions targeted at the SCN in rodents translate into a phase shift of the rest-activity rhythm of the animals. In rodents, the physical destruction of the SCN results in a complete suppression of the rest–activity rhythm, while the transplantation of SCN restores circadian rhythmicity. These experimental facts clearly demonstrate the dependency of this rhythm upon SCN function [66].

In man, the rest–activity rhythm is considered and used as a marker of the circadian timing system in isolation studies, in phase shift studies and in psychiatry [67]. The rest–activity rhythm can be measured by using
a small size instrument worn on the wrist known as an Actigraph. This type of monitoring is totally non-invasive therefore; there is no restriction to its use in cancer patients. The easy recording of rest–activity has further supported its use as a reference rhythm for the circadian timing of medications and for the evaluation of circadian clock function.

The Molecular Circadian Clock: Control of Cell Cycle, Apoptosis and Repair: Recently, the complex machinery of the molecular clock was shown to exert a negative control on the transcriptional activity of some key genes involved in cell cycle regulation, which suggested that cell proliferation could be regulated by the circadian clock. Circadian rhythms have been extensively reported for cell cycle phase distribution in healthy or malignant mammalian tissues [68-71]. Two recent studies have further identified c-myc, p53 and wee1 as being clock-controlled genes [72, 73]. C-myc and wee respectively promote cell cycle progression from G1 to S and from G2 to M. C-myc can also exert proapoptotic effects through p53-dependent or independent pathways [74-76].

Many other cell cycle-related genes display 24-h rhythms in mRNA and/or protein expression in healthy tissues from rodents and/or humans, also equipped with molecular clock components. This is the case for the expression pattern of genes which control cell cycle checkpoints, such as cdk2, cyclins A, B1, D, E or mdm2, or which regulate apoptosis, such as gadd45a, bcl2 and bax [77]. The clock genes cry are members of the photolyases family, which is involved into the repair of UV DNA damage, indicating a close link between both the systems [78].

Chronotherapy and Cancer Treatment: The main obligation on treatments of cancer is to maximise the cytotoxicity to cancer cells and at the same time trying to avoid drug-resistance. Anti-cancer drugs work by targeting actively proliferating cells. They act on a specific part of the cell cycle and focus on that phase to try and kill the cell. However, cancer cells and normal cells follow the same replication pathway so there is no way to stop the cancer drugs from attacking normal cells. Therefore, a balance must be reached between the drug’s ability to target cancer cells effectively and its negative effects on the body. This is known as the ‘therapeutic index’ [79]. Cancer chronotherapy works to improve this index so that there are less negative effects on the body and there is faster targeting of the cancer cells.

The main dogma behind chronotherapy is that it takes advantage of the fact that normal cells follow the circadian rhythm when it comes to the cell cycle. As a result, at a particular time of the day, the phase of the cell cycle in normal cells can easily be determined. However, tumour cells do not follow the 24 hour circadian rhythm and are more often at a different phase of the cell cycle than normal cells. It has been well recognized that the DNA synthesis rhythm in healthy cells is in a phase opposite to tumour cells [80]. Therefore, the treatment is given at the time of day when there will be least damage to host tissues.

A number of key factors determine the efficiency of a cancer drug such as absorption, distribution, intracellular metabolism and elimination. These variables are more favourable at different times of the day because of the physiological changes due to circadian rhythm. For example, the blood supply to the tumour may vary according to circadian rhythm and therefore, by determining when there is maximal blood flow to the tumour, treatment can be given so that it maximises the cytotoxic effect. It has also been shown that the best time for treatment is early in the morning, because most of the target cells are very vulnerable at this time.

The effectiveness of chronotherapy depends to a certain extent upon the degree of coupling between the central pacemaker and the tumour’s cell cycle. In slow growing tumours, there is a greater degree of coupling and hence, chronotherapy is a lot more effective as it is possible to manipulate the patient’s circadian clock to aid treatment. For example, circadian rhythm can be regulated or deregulated depending upon the desired effect through light therapy as light is the most important environmental prompt for the central pacemaker. However, further controllable stimuli could include meal times, melatonin or glucocorticoid administration.

Cell-cycle events are coordinated along the 24-h period in healthy bone marrow, gut and skin, which are three frequent targets for the toxicity of cancer treatments. The proportions of S- and G2/M-phase cells increase by approximately 50% in the second half of the dark phase of the light–dark cycle, whereas G0/G1 cells predominate during the light phase in the total bone marrow of male B6D2F1 mice. In this tissue, Bcl-2 protein levels triple over the 24- h period, with a maximum levels being reached at early light [81]. An opposite pattern characterizes the proapoptotic protein BAX, which has a five-fold 24-h change and a peak at Zeitgeber time [82]. The temporary arrest of cycling cells in G0/G1 and the high Bcl-2 and low
Table 3: Various patents in the field of chronotherapy

<table>
<thead>
<tr>
<th>Technology</th>
<th>Patent No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantable electromechanically driven device</td>
<td>U.S. Pat. No. 4,003,379</td>
</tr>
<tr>
<td>Flexible system for timed controlled or position controlled drug delivery system</td>
<td>U.S. Pat. No. 7048945</td>
</tr>
<tr>
<td>Three Dimensional Printing® (3DP) technology</td>
<td>U.S. Pat. No. 5,490,962</td>
</tr>
<tr>
<td>Self-powered medication systems</td>
<td>U.S. Pat. No. 5,692,027</td>
</tr>
<tr>
<td>Implantable infusion device</td>
<td>U.S. Pat. No. 4,003,379</td>
</tr>
<tr>
<td>Pulsatile delivery</td>
<td>U.S. Pat. No. 6635277</td>
</tr>
<tr>
<td>Pulsatile technology</td>
<td>U.S. Pat. No. 5914134</td>
</tr>
<tr>
<td>Self-powered medication systems</td>
<td>U.S. Pat. No. 4,146,029</td>
</tr>
<tr>
<td>Pulsatile technology</td>
<td>U.S. Pat. No. 6217904</td>
</tr>
<tr>
<td>Microchip drug delivery devices</td>
<td>U.S. Pat. No. 5,797,898</td>
</tr>
<tr>
<td>Beads</td>
<td>U.S. Pat. No. 5439689</td>
</tr>
</tbody>
</table>

BAX levels during the light span when mice rest, help to explain the best circadian timing for the tolerability of cancer treatments such as Irinotecan and Docetaxel in male B6D2F1 mice [83, 84]. The circadian control of drug metabolism and detoxification also profoundly modifies cell exposure to these medications, whose molecular targets are usually clock regulated. For example, the increased detoxification of 5-FU during the light span results from the circadian peak in dihydropyrimidine dehydrogenase activity in liver and other healthy cells [85]. The outcome is reduced proportions of S-phase cells in bone marrow, gut and skin, as a mechanism for improved circadian tolerability [86]. Both the transcription and activity of thymidylate synthetase (TS), which produces a unique de novo source of thymidylate, are linked to the early S-phase in proliferating tissues [87]. Bone marrow TS activity peaks near the mid-dark phase and this coincides with the greatest haematological toxicity of 5-FU in female CD2F1 mice. Whereas circadian phase I and II metabolism partly determine Irinotecan pharmacology over the 24-h period, topoisomerase I (Topo I; the main protein target of this drug) is mostly active during the late S-phase [88]. Thus, the circadian gating of the cell cycle and, possibly, the direct control of Topo I by the molecular clock contribute to better haematological tolerability of Irinotecan during the rest span of male ICR mice.

Circadian dosing time influences the extent of toxicity of more than 30 anticancer drugs and it has been shown in animal models that the survival rate varies by at least 50% depending on when a ‘lethal dose’ of drug is given. This may be due to the fact that the cellular repair mechanisms, such as nucleotide excision, are subject to circadian regulation and many key genes are associated with xenobiotic metabolism and transport [89].

Circadian Clocks and Cancer Prognosis: Circadian rhythm is regarded as a prognostic (Predictive) indicator of survival. Many studies indicate that patients with metastatic colorectal or breast cancer may exhibit circadian rhythm abnormalities, which either reflect aggressive disease or contribute to disease prognosis via mechanisms that need to be explored further [88]. These studies suggest that altered circadian clocks may contribute to the acceleration of cancer growth [90]. Another study has shown that hormonal circadian rhythms were altered in women at high risk of breast cancer compared with women at low risk of this disease [91]. Table 3 shows the various patents in the field of chronotherapy.

CONCLUSION

The role of circadian clocks in cancer prevention and chemotherapy has long been researched and substantiated. Cancer chronotherapy is currently in practice and it is being studied intensely so as to find better and superior ways for determination of optimal time frame for treatment. The clinical trials show affirmative emergence in the field of cancer chronotherapy and hopefully in the near future, the use of the circadian cycle will help to optimize cancer therapy and minimize the harmful effects.

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REFERENCES


