FACULTY OF
PHARMACEUTICAL SCIENCES

Chronotherapy in chronically ill patients

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A Master dissertation for the study programme Master in Drug Development

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ABSTRACT

BACKGROUND: Universal biological patterns, which are periodic oscillations of molecular, behavioural or physiological processes, are present in all living organisms. Cycles with a 24-hour schedule are called circadian rhythms. The main purpose of these rhythms is maintaining living processes by maximizing metabolism and energy utilization.

OBJECTIVES: The main objective of this master thesis is the investigation of the pathogenesis of diseases with established oscillatory rhythms and the exploration of chronotherapy as a potential treatment approach for hypertension and rheumatoid arthritis. The thesis also aims to examine the influence of circadian rhythms on the pharmacokinetics and pharmacodynamics of medication to achieve the optimal drug concentrations at the intended site of action within the body at the correct time to achieve the best treatment response.

METHODS: Different literature websites like PubMed and Web of Science were used with the goal of discovering an answer to the research questions. A PK/PD model was used to investigate the impact of chronotherapy on the treatment of rheumatoid arthritis.

RESULTS: Administration of antihypertensive drugs at specific times of the day, especially bedtime, resulted in improved blood pressure control compared to conventional therapy. These findings provide evidence for the effectiveness of chronotherapy in optimizing blood pressure control and reducing cardiovascular risk. Timing the administration of anti-rheumatic drugs to coincide with the circadian rhythm and the inflammatory processes associated with rheumatoid arthritis has also demonstrated improved disease control. Specifically, administering drugs in the early morning, when the immune response is heightened, can enhance the effectiveness of treatment.

CONCLUSION: Implementing chronotherapy with optimal drug timing, such as taking antihypertensive drugs and anti-rheumatic drugs at night, improves treatment outcomes and minimizes side effects in various conditions. Further research and the use of PK/PD models are necessary to further develop chronotherapy in circadian rhythm-based treatment.

ABSTRACT

ACHTERGROND: Universele biologische patronen, die periodieke oscillaties van moleculaire, gedrags- of fysiologische processen zijn, komen voor in alle levende organismen. Cycli met een 24-uursschema worden circadiaanse ritmes genoemd. Het belangrijkste doel van deze ritmes is het handhaven van levensprocessen door het maximaliseren van metabolisme en energiegebruik.

OBJECTIEF: Het hoofddoel van deze masterthesis is het onderzoeken van de pathogenese van ziekten met gekende oscillatoire ritmes en het verkennen van het gebruik van chronotherapie als mogelijke behandelingsoptie voor hypertensie en reumatoïde artritis. Deze thesis heeft ook als doel om de impact van circadiaanse ritmes op de farmacokinetiek en farmacodynamiek van medicatie te onderzoeken om optimale concentraties van het geneesmiddel op de beoogde plaats van werking in het lichaam te bereiken op het correcte tijdspunt zodat het beste antwoord op de therapie bekomen wordt.

METHODEN: Verschillende literatuurwebsites zoals PubMed en Web of Science werden gebruikt met als doel een antwoord te vinden op de eerdergenoemde onderzoeksvragen. Een PK/PD-model werd gebruikt om het effect van chronotherapie op de behandeling van reumatoïde artritis te onderzoeken.

RESULTATEN: Het toedienen van antihypertensiva op specifieke tijdstippen van de dag, met name voor het slapengaan, leidde tot een betere bloeddrukcontrole en een daling van het cardiovasculair risico in vergelijking met conventionele therapie. Het timen van de toediening van anti-rheumatica om samen te vallen met het circadiaanse ritme en de ontstekingsprocessen heeft ook aangetoond dat de ziektecontrole verbetert. Specifiek het toedienen van geneesmiddelen in de vroege ochtend, wanneer de immuunrespons verhoogd is, kan de effectiviteit van de behandeling versterken.

CONCLUSIE: Het implementeren van chronotherapie met optimale timing van medicatie, zoals het innemen van antihypertensiva 's en anti-rheumatica in de avond, verbetert de behandelresultaten en minimaliseert bijwerkingen bij verschillende aandoeningen. Verder onderzoek en het gebruik van PK/PD-modellen zijn noodzakelijk om chronotherapie verder te ontwikkelen in behandelingen gebaseerd op het circadiaanse ritme.

ACKNOWLEDGMENT

During the second semester of my first master year, I conducted a literature search on chronotherapy in chronically ill patients.

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LIST OF ABBREVIATIONS

ABPM	Ambulatory Blood Pressure Monitoring
ACEI	Angiotensin-Converting Enzyme Inhibitors
ADME	Absorption, Distribution, Metabolism, Excretion
ARB	Angiotensin II Receptor Blockers
AUEC	Area Under Effect Curve
BMAL1	Brain Muscle Arnt Like 1
BP	Blood Pressure
CCB	Calcium Channel Blockers
CCS	Cumulative Cortisol Suppression
CK1δ/ε	Casein Kinase 1 delta/epsilon
CKD	Chronic Kidney Disease
CLOCK	Circadian Locomotor Output Cycles Kaput
COER	Controlled Onset Extended Release
COVID-19	Corona Virus Disease 2019
CRY	Cryptochrome
CVD	Cardiovascular Disease
DBP	Diastolic Bloop Pressure
DLMO	Dim Light Melatonin Onset
DMARD	Disease-Modifying Antirheumatic Drugs
DNA	Desoxyribonucleic acid
GITS	Gastrointestinal Therapeutic System
HDL	High Density Lipoprotein
HPA	Hypothalamic-Pituitary-Adrenal
IFN-γ	Interferon gamma
IL-6	Interleukin 6
IV	Intravenous
LDL	Low Density Lipoprotein
MEC	Minimal Effective Concentration
MTC	Minimal Toxic Concentration

NSAID	Non-Steroidal Anti-Inflammatory Drugs
PBPK	Physiologically Based Pharmacokinetic
PD	Pharmacodynamic
PER	Period
PK	Pharmacokinetic
PKPD	Pharmacokinetic-Pharmacodynamic
PopPK	Population Pharmacokinetic
P-PPK	Population-Phase-Model-of-Oscillator-interactions
RA	Rheumatoid Arthritis
RAAS	Renin-Angiotensin-Aldosterone System
RCT	Randomized Controlled Trials
RevRE	Rev-erb respons elements
ROR	Retinoic acid related-Orphan Receptors
RORE	Retinoic acid related-Orphan Receptors elements
SBP	Systolic Blood Pressure
SCN	Suprachiasmatic Nucleus
TNF-α	Tumor Necrosis Factor alpha

1. INTRODUCTION

1.1. CIRCADIAN RHYTHMS

Universal biological patterns, which are periodic oscillations of molecular, behavioural or physiological processes, are present in all living organisms. The study of biological rhythms is known as chronobiology. Depending on the duration of the period (τ), biological rhythms can be categorized into ultradian, circadian and infradian rhythms. The period is the amount of time required to complete one cycle and can be classified as short (τ < 0.5h), intermediate (0.5h < τ < 6 days) or long (τ > 6 days) [1],[2]. Cycles that are shorter than 20 hours, like the cycle of cortisol secretion, are known as ultradian rhythms. Cycles with a 24-hour schedule are called circadian rhythms, where the sleep-wake cycle is an illustration of this. Infradian rhythms are those that have a frequency higher than 28 hours. The menstrual cycle is a well-established infradian rhythm in humans [3],[4]. Rhythmicity is exhibited by a variety of physiological processes, such as cycles of sleep and wakefulness, nutrition, endocrine secretions, hepatic metabolism, body temperature, neurotransmitters and second messengers, plasma proteins, enzymes, cardiovascular activity, renal function and others [6],[7].

When individuals are exposed to a lack of light and dark cycles, such as in the case of completely blind individuals or those constantly in very low light environments, their circadian rhythms undergo a process called desynchronization from the 24-hour day. Consequently, internal biological rhythms become decoupled from external/environmental cues, such as light signals, temperature, feeding, oxygen levels and activity. As a result, there can be a gradual shift or drift of sleep-wake patterns and other physiological processes away from the conventional 24-hour day [5].

1.2. THE CIRCADIAN CLOCKS

Circadian rhythms are regulated by a complex system of circadian clocks, which consist of a hierarchical network of central and peripheral clocks. This network is responsible for the

generation, maintenance and coordination of circadian rhythms. The circadian clock in the body runs for roughly 24 hours a day under constant environmental conditions [6]. The main purpose of the circadian clocks is to maintain living processes by maximizing metabolism and energy utilization, so the organism is able to anticipate, adapt and react to variations in environmental cues [5].

Both diurnal and circadian relate to daily rhythms, but they refer to different aspects of those rhythms. Diurnal refers to patterns that recur over the course of a single day, such as the pattern of sleep and wake [8]. They are influenced by external cues as well as internal factors like metabolism and hormone levels. Circadian, on the other hand, refers to biological rhythms that recur over approximately 24-hour cycles, independent of external cues [9]. The suprachiasmatic nucleus (SCN) in the hypothalamus of the brain serves as the master pacemaker of the circadian clock system. The SCN receives and integrates environmental cues to establish the body's internal timekeeping [6]. The circadian clock system operates through a series of interconnected molecular feedback loops involving specific clock genes and their protein products. These molecular components form the basis for the generation and maintenance of circadian rhythm processes, referred to as 'output' in Figure 1.1. [12].

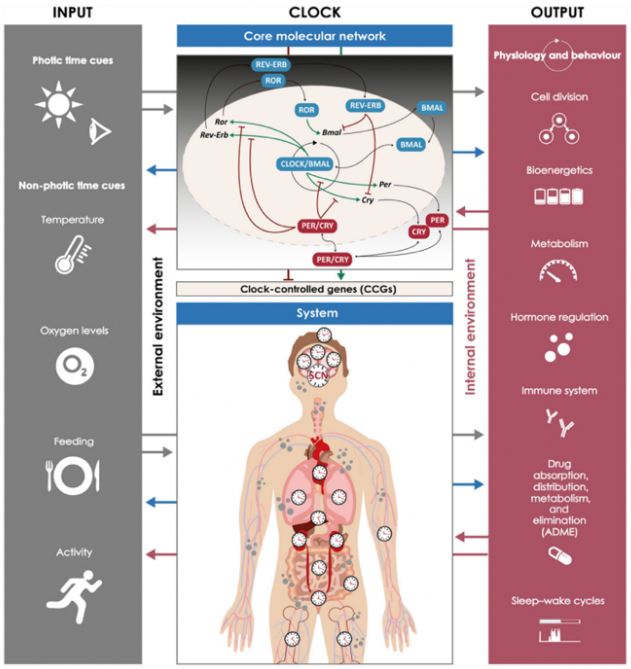

Figure 1.1. The circadian clock system [12].

1.2.1. The master/central clock

The central pacemaker known as "the master clock" that resides in the SCN controls the hierarchy of the mammalian timing network [13]. These suprachiasmatic nuclei are pairs of neuronal clusters, that communicate with each other and with other hypothalamic structures, situated in the anterior hypothalamus. It is shown that a bilateral ablation of the SCN compromise the circadian control of behavioural, endocrine and metabolic rhythms. Surgical removal of the SCN causes arrhythmia in a number of processes, while transplanting the area back from another donor animal restores the rhythmicity [14]. This provides evidence that the master clock is present in the SCN. The term "peripheral clocks" refers to additional circadian oscillators that are regulated by the main master clock and are found in various organs and regions of the brain. The circadian clock of SCN neurons and peripheral cells is identical at the molecular level. However, there is a significant difference in how they synchronize (Section 1.2.2) [15].

Direct innervation from the retina facilitates the master clock's synchronization with light and dark signals. The SCN, which synchronizes and influences the circadian clock, is shown to be light-entrained (Figure 1.2.) [1],[16],[17]. The retinohypothalamic tract facilitates the transmission of light information from photosensitive retinal ganglion cells to the SCN in the hypothalamus. The SCN then delivers hormonal and autonomic outputs to the pituitary gland, hypothalamic system and central autonomic control system that control the endocrine system, dark-light cycles and sympathetic and parasympathetic system, respectively [18],[19].

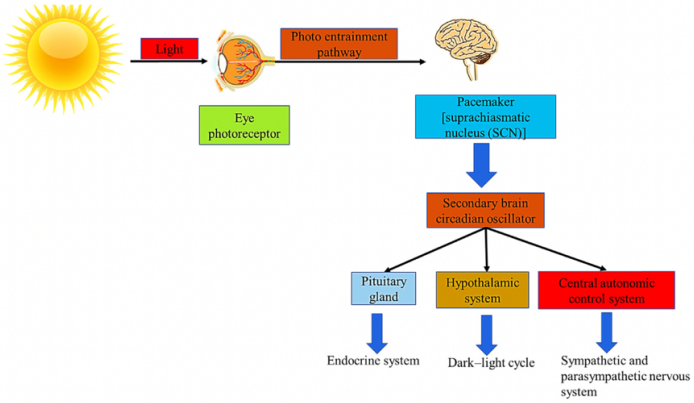

Figure 1.2. Demonstration of the SCN entrainment [1].

The circadian clock system operates through a series of interconnected molecular feedback loops involving specific clock genes and their protein products. These molecular components form the basis for the generation and maintenance of circadian rhythms. The two primary feedback loops involved are the transcription-translation feedback loop and the post-translational feedback loop. (Figure 1.3.) [6],[16]. Circadian Locomotor Output Cycles Kaput (CLOCK) and Brain Muscle Arnt Like 1 (BMAL1) are transcription factors that comprise the feedback loop's positive arm. The feedback loop's negative arm consists of the elements period (Per) and cryptochrome (Cry). Regardless of if the animal is nocturnal or diurnal, this basic system is attached to the light-dark cycle, with CLOCK:BMAL being significant during the light phase and Per:Cry being significant during the dark period [18].

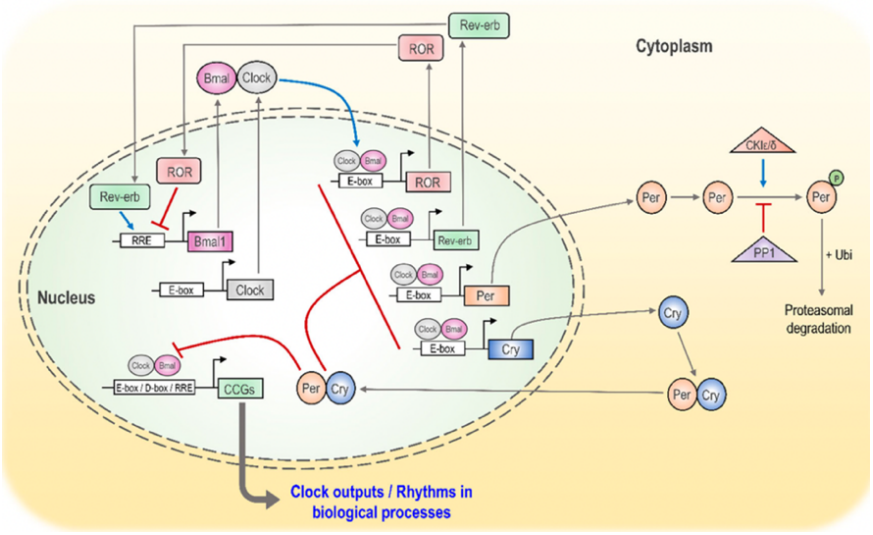

Figure 1.3. The transcription-translation, post-translational and ROR/Rev-erb feedback loops influenced by activators, repressors, proteins and clock genes [6].

CLOCK and BMAL1 heterodimerize during the light period, which is followed by a translocation to the nucleus where they attach to DNA at specific sites called E-box elements. These heterodimers activate the transcription of Per and Cry genes. The Per and Cry genes are transcribed into mRNA, which is then translated into Per and Cry proteins. Accumulated Per and Cry proteins move back into the nucleus and inhibit the activity of CLOCK-BMAL1 (heterodimer), forming a negative feedback loop that decreases their own gene expression. Over time, Per and Cry proteins are degraded, releasing the inhibition on CLOCK and BMAL1 and allowing a new cycle to begin. [6],[20]. The post-

translational feedback loop exists of phosphorylation and degradation. Proteins such as Casein Kinase 1 delta/epsilon (CK1δ/ε) phosphorylate Per and Cry proteins, marking them for degradation. During the circadian cycle, the levels of CK1δ/ε decrease, leading to dephosphorylation and stabilization of Per and Cry proteins. With the next circadian cycle, CK1δ/ε levels increase again, initiating the phosphorylation and degradation of Per and Cry proteins.

The circadian clock system involves an additional feedback loop that includes two additional clock genes: ROR and Rev-erb. This loop acts in conjunction with the transcription-translation and post-translational feedback loops mentioned earlier. ROR and Rev-erb genes are activated by CLOCK-BMAL1 heterodimers binding to ROR response elements (ROREs) and to Rev-erb response elements (RevREs) in their promoter regions. Activated ROR and Rev-erb genes are transcribed into mRNA, which is then translated into proteins. These proteins translocate back to the nucleus where ROR proteins enhance BMAL1 transcription, while Rev-erb proteins inhibits it [4],[16].

These three interconnected feedback loops, the transcription-translation loop, post-translational loop and ROR/Rev-erb loop, work together to regulate and maintain the circadian clock system. The transcription-translation loop controls the expression of clock genes, the post-translational loop modulates the stability and degradation of clock proteins and the ROR/Rev-erb loop adds an additional layer of regulation to fine-tune the clock system.

1.2.2. The peripheral clocks

The peripheral clocks are secondary circadian clocks found in various organs and tissues outside of the central pacemaker in the SCN. While the SCN serves as the master pacemaker, coordinating the overall circadian rhythm, peripheral clocks are responsible for maintaining local circadian rhythms within specific tissues or organs, such as liver, kidney, adipose tissue, heart, lung, skeletal muscles and others [10]. The peripheral clocks are composed of similar molecular components as the central clock in the SCN. They consist of clock genes (such as CLOCK, BMAL1, Per and Cry) and their protein products that

interact in feedback loops, like the transcription-translation and post-translational feedback loops described earlier.

The synchronization of peripheral clocks with the central pacemaker and the external environment is achieved through a process called entrainment. The SCN receives light signals and other environmental cues and communicates with peripheral clocks through neural and hormonal pathways, thereby coordinating their phase and timing [4],[22]. Each peripheral clock regulates local gene expression and activity to optimize organ-specific functions in a time-of-day-dependent manner. These local circadian rhythms influence various physiological processes within specific tissues or organs, including metabolism, hormone secretion, cellular repair, immune response and many others. For example, the liver clock regulates metabolic pathways involved in nutrient utilization, while the adrenal gland clock controls the release of stress-related hormones [21],[23].

1.2.3. Integration of central and peripheral clocks

The integration of central and peripheral clocks involves the coordination and communication between the master pacemaker in the brain and clocks in organs and tissues. As stated before, the SCN communicates with peripheral clocks through neural and hormonal signals. It receives light information via retinal ganglion cells, allowing it to synchronize the timing of both central and peripheral clocks. Systemic cues, such as temperature and behaviour, also play a role. This coordination ensures that physiological processes in the body are properly timed and aligned with the external environment. Disruptions in this integration can lead to circadian rhythm disruptions and related health issues. Maintaining a synchronized circadian system is important for overall well-being [18].

1.3. CIRCADIAN RHYTHM DISRUPTION

1.3.1. The physiology of disruption

A disorder, dysregulation or issue that impairs circadian processes is known as a circadian disruption. Disruptions in human physiology can lead to a variety of physiological illnesses such as sleep disorders, perturbations in circadian rhythms, alterations in body weight and

perturbed metabolic processes [26]. Changes in circadian timing are frequently linked to sleep-wake disruptions, which also have a negative impact on health. As illustrated by immunologic, cardiometabolic, neurodegenerative and mental diseases, the connection between human disease and circadian cycles can lead to a vicious loop between illness and circadian disturbance [27].

1.3.2. Causes

Most of the population has become what is known as a "24-hour society," which is compelled to operate outside of its normal temporal scope, because of work, care and even education routines. According to some figures, about half of people in industrialized countries have circadian rhythms that are out of sync with the rigid everyday schedules they are subjected to [29]. Social jetlag, which refers to the misalignment between individuals' internal biological clocks and external societal demands, can have various consequences on health. One aspect contributing to social jetlag is the presence of diverse temporal inclinations or chronotypes in the population. These chronotypes range from extreme night owls to extreme morning larks, showcasing the interindividual diversity in temporal preferences. The internal desynchronization between the master biological clock, and external rhythms can lead to several health issues. These issues encompass disrupted sleep patterns, metabolic dysregulation and even mental conditions. For instance, when individuals experience jetlag due to travel across time zones or work night shifts, their internal time becomes misaligned with the external clock time, resulting in social jetlag. Disruptions caused by electromagnetic waves, unbalanced nutrition, altered gene expression and exposure to artificial light can also play a role in throwing off individuals' biological clocks and exacerbating social jetlag (Figure 1.4.) [9],[30].

1.3.3. Circadian rhythms associated diseases

Over time, clock disruption can cause several serious diseases, which can then feedback and further abrogate rhythms. Neurodegeneration, cardiovascular diseases (CVD), metabolic syndrome, diabetes, cancer, obesity, infertility, depression and others are examples of these disorders (Figure 1.4.) [1],[8]. Asthma, systemic lupus, rheumatoid

arthritis and other inflammatory auto-immune illnesses exhibit diurnal variations in symptom intensity. Furthermore, disruption of the host circadian clock can result in an increase in the replication and spread of pathogens, proving that circadian rhythms can have an impact on the severity of acute infections. Such intricate relationships have been assessed as being particularly relevant during the coronavirus disease 2019 (COVID-19) pandemic [6].

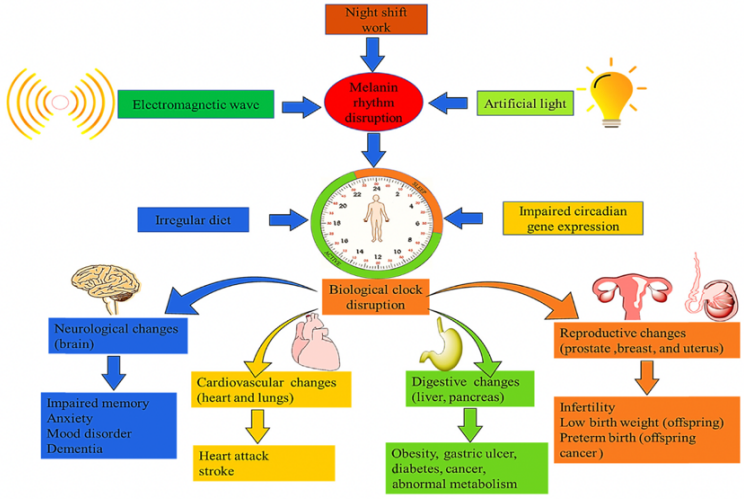

Figure 1.4. Causes and consequences of circadian disruption [1].

1.3.4. Circadian rhythms based-therapies

Various treatments have been suggested for conditions associated with circadian rhythm disruption, including phototherapy, scheduled sleep, modification of diet and lifestyle, wakefulness-promoting medications, as well as chronobiotics [17]. These treatments are called ‘clock modulators’. The creation of therapy methods to alleviate some of the negative effects of improper circadian timing is being driven by the acute and potential long-term effects of circadian rhythm disorders [5].

The best therapy options at this time is phototherapy because it has been shown that this phase-shifts the timing of the human circadian rhythm [32]. The circadian timing system can be advanced or delayed by light at the right moment. It is obvious that the time of light delivery, along with the strength of light, its period and its wavelength, all affect how much light can change the clock [33]. The most efficient way to phase-shift the human circadian

clock is with short-wavelength light (420–480 nm) [5]. Besides, wakefulness-promoting drugs have been used to lessen some of the negative effects of jetlag and shift work-related circadian misalignment, such as short-acting benzodiazepines, modafinil and caffeine.

A class of medications known as "chronobiotics" are those that can retrain circadian cycles by changing the phase of the circadian clock. Melatonin, a hormone that coordinates diurnal rhythms, is of particular importance. Melatonin helps people fall asleep and encourages restful slumber [34]. Oral melatonin has been shown to have the ability to cure insomnia, jetlag, shift work and sleep problems linked to the circadian cycle. Melatonin minitablets have been tried on children and teenagers with neurogenetic disorders and autistic spectrum disorder and have proven to be effective and safe for the therapy of sleeplessness [35].

Phytochemicals, another clock modulator, appeared to have the benefit of modifying circadian cycles without producing negative side effects [19]. It has been proposed that resveratrol works well as a clock modulator. Resveratrol is a natural compound found in certain plants, grapes, blueberries, peanuts and some herbs. It belongs to a class of substances called polyphenols, which function as antioxidants and aid in defending cells against injury from free radicals. Resveratrol supplementation enhanced the rhythmicity of blood sugar, plasma insulin and the overall metabolic status of patients with a high-fat diet-induced illness. Additionally, the clock genes (CLOCK, BMAL1, Per and Cry) periodic expression is altered by resveratrol [37]. Numerous other drugs target key regulators of the central clock, like Rev-erb, a key regulator of BMAL1's circadian expression. The nuclear receptors Rev-erb may be targeted to increase wakefulness, diminish rapid eye movement, lower anxiety-like behaviour and control the structure of sleep and emotional responses [19],[22].

1.4. CHRONOPHARMACOLOGY

Chronopharmacology is a branch of pharmacology that focuses on understanding the effects of drugs in relation to the body's circadian rhythms and biological clock. It examines

how the timing of drug administration can influence their effectiveness, safety and tolerability [38]. The body's physiological processes, including drug metabolism, absorption, distribution and elimination, exhibit circadian variations. These variations can affect the pharmacokinetics (what the body does to the drug) and pharmacodynamics (what the drug does to the body) of drugs [7]. Chronopharmacology considers the concept of chronotherapy, which involves administering drugs at specific times of the day to optimize therapeutic outcomes. By aligning drug administration with the body's natural circadian rhythms, chronopharmacology aims to improve drug efficacy, minimize side effects and enhance patient outcomes [3].

1.4.1. Chronopharmacokinetics

The study of periodic, time-predictable variations in drug pharmacokinetics is known as chronopharmacokinetics. This refers to the timing-related changes in drug absorption, distribution, metabolism and elimination. The rates at which drugs are processed by the body can vary throughout the day due to fluctuations in enzyme activity, blood flow, glomerular filtration rate and others (Figure 1.5.) [15],[1].

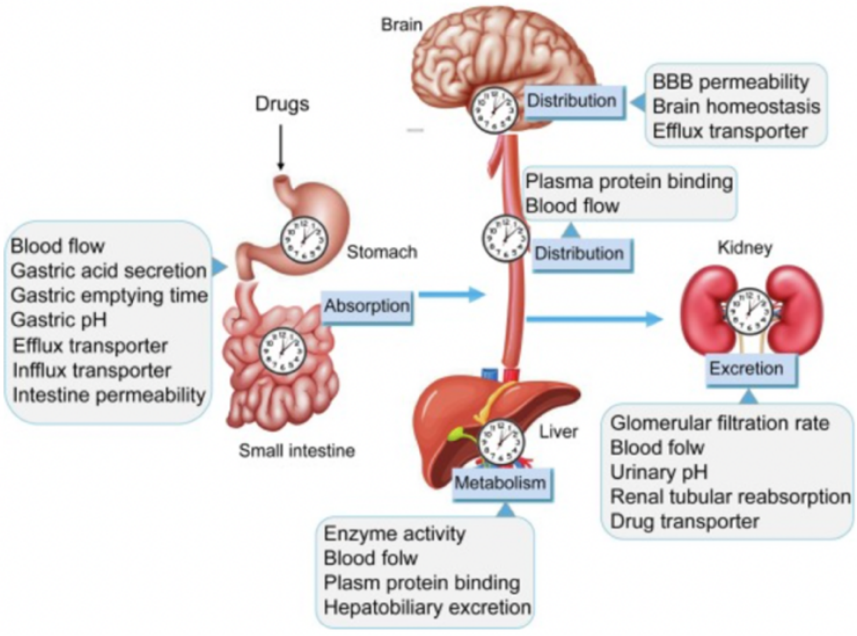

Figure 1.5. A conceptualization of the development of circadian-dependent physiological variables in oral drug disposition [21].

1.4.1.1. Absorption

It has been established that circadian rhythm affects the absorption of oral drugs because changes in the circadian rhythm affect the gastric pH, intestine permeability, secretion of acids, gastric emptying time, motility, transporters in the gut and gastrointestinal blood flow (Figure 1.5.) [6]. For instance, based on the physicochemical features of the drug, diurnal variations in gastric pH may influence drug ionization [38]. Also, the length of time before the stomach empties is a significant component in the absorption of drugs. Gastric emptying rates were examined between morning and evening, and it was discovered that the evening meal's gastric emptying half-life ($t_{1/2}$) was considerably prolonged compared to morning meals. The extension of gastric emptying time post-dinner can cause a delay in the realization of optimal plasma concentrations of certain drugs. Lipophilic drugs (such as cyclosporine, tacrolimus and propranolol) have quicker absorption rates in the morning compared to the evening, and this difference may be explained by the physicochemical characteristics of the drug. Also, non-steroidal anti-inflammatory drugs (NSAID) absorption in people varies according to the circadian cycle. The maximal concentration (C_{max}) is higher after morning drug administration due to a faster stomach emptying time and a higher gastrointestinal perfusion in the morning [6],[17],[15].

Biological cycles not only impact medication absorption through the oral pathway but also through other routes like ocular permeability, transdermal permeability and pulmonary permeability [1].

1.4.1.2. Distribution

Physiological factors such as the blood flow rates to the organ/tissue, the presence of drug transporters, the tissue volumes, red blood cell partitioning and protein binding in plasma and tissues have a significant impact on the volume of distribution of a drug in plasma/blood and various tissues [6]. The sympathetic and parasympathetic nervous systems are two regulatory factors that influence blood flow. These systems exhibit circadian rhythmicity, with the sympathetic system demonstrating a predominance of daily effects. Thus, a

possible variation in drug distribution according to dosing time may be explained by local tissue blood flows. Daytime increases the blood flow, while at night it decreases.

Because of the circadian cycle, the liver's function changes. Therefore, the levels of circulating proteins (albumin and globulins) change throughout day and night. Most human plasma proteins, such as albumin and α_1-glycoprotein, increase during the day and are at their peak at noon. When a drug is bound to proteins, it is considered unactive. As a result, daily variations in a drug's plasma protein binding have been observed. For instance, the plasma free phenytoin and valproic acid concentrations are at their maximum between 2 - 6 a.m., diazepam and carbamazepine have the opposite effect. There levels are at their lowest in the morning due to significant protein binding (and metabolism rhythmicity, Section 1.4.1.3.). Transcortin, a blood-borne corticosteroid transporter protein, binds to prednisolone and other endogenous hormones with circadian cycles. The binding capacity is lowest at night and rises further after 4:00 a.m., reaching its peak after 4:00 p.m. These results imply that the morning achieve high enough concentrations of free plasma corticosteroids [1]. According to studies, young and healthy adults demonstrate less diurnal fluctuation in plasma proteins than healthy senior adults (75 years). the aging process is associated with alterations in plasma protein levels, particularly during nighttime rest. This can result in an increased free fraction of drugs that are typically bound to plasma proteins, potentially influencing drug activity and response in older adults [15].

Drugs with a limited volume of distribution, a strong protein-binding capacity and/or small therapeutic indices may be impacted by modifications in circadian rhythm from a toxicological perspective [15].

1.4.1.3. Metabolism

The liver plays a crucial role in the metabolism of xenobiotics (foreign substances, including drugs) in animals. However, extrahepatic metabolism also occurs in various tissues such as the brain, kidney, gut and lung. Xenobiotic metabolism involves three types of enzymes that facilitate the transformation of these substances into more easily excretable forms [6]. Phase I enzymes, such as cytochrome P450, are responsible for breaking down

xenobiotics. These enzymes initiate chemical modifications, making the substances more susceptible to further processing. Phase II enzymes, including epoxide hydrolases and glutathione-S-transferases, then convert the modified substances into water-soluble forms, facilitating their elimination from the body. Phase III reactions further refine these forms, preparing them for removal through urine, faeces and respiration [15].

The activity of xenobiotic-metabolizing enzymes exhibits circadian rhythmicity, meaning it varies depending on the time of day. This regulation of drug metabolism throughout the day influences both drug efficacy and toxicity. Examples of drugs that undergo circadian-dependent metabolism include diazepam, carbamazepine and phenytoin, as mentioned before. Diazepam and carbamazepine are primarily metabolized by liver enzymes that exhibit diurnal variations in their activity levels. These enzymes can be influenced by circadian rhythms, leading to fluctuations in drug metabolism throughout the day. The enzymes responsible for metabolizing diazepam and carbamazepine are higher during the day, resulting in more rapid breakdown and elimination of these drugs. As a result, the plasma levels of diazepam and carbamazepine tend to be lower in the morning. Phenytoin, on the other hand, is primarily metabolized by the liver enzyme CYP2C9, which does not show significant diurnal variation in its activity. Therefore, the highest plasma levels of phenytoin are not necessarily concentrated in the morning [15].

1.4.1.4. Excretion

By affecting how medications are metabolized in the liver and gut and how they are eliminated through bile movement and via the urine, the circadian timing system has a significant impact on how toxic substances' concentrations change over time [15].

The release of lipids, bile acids and xenobiotics into the bile is regulated by the diurnal cycle. In humans, the production of bile acids strictly follows a circadian rhythm. This rhythmic pattern is governed by the activity of the enzyme cholesterol-7-hydroxylase (CYP7A1), which is responsible for converting cholesterol into bile acids. The expression of CYP7A1 is primarily controlled by a protein called Rev-erb. Moreover, the liver exhibits

a diurnal rhythm in the expression of most genes that encode the transporters involved in bile release [17].

The clearance of medications can be influenced by variations in urinary pH, which change throughout the day based on the body's circadian rhythm. The pH of urine is typically basic during the day and acidic at night. This pH difference can affect the elimination of certain drugs. Weakly basic medications are eliminated more during the day when urine is basic, while weakly acidic medications are excreted more at night when urine is acidic. The timing of drug elimination in urine is dependent on the body's circadian rhythm. Sulfasalazine urine elimination is an example of a circadian time-dependent drug. For intravenous drugs, evening dosing is often times associated with lower clearance and higher exposure [4]. The parameter that changes the most during intravenous drug dosing in the evening versus other times of day is the drug clearance rate.

1.4.2. Chronopharmacodynamic

Chronopharmacodynamics refers to the study of how the response of the body to drug or medication varies according to the time of administration, considering the body's circadian rhythms and biological clock. The efficacy and pharmacological effects of drugs can be influenced by the body's natural rhythms, including fluctuations in receptor sensitivity, enzyme activity and signaling pathways throughout the day. Chronopharmacodynamics aims to understand how these variations in drug response occur and how they can be utilized to optimize therapeutic outcomes [3]. Chronopharmacodynamics also considers the concept of chronotherapy, which involves administering drugs at specific times to enhance their efficacy and minimize side effects. By aligning drug administration with the body's natural rhythms, it is possible to maximize the drug's therapeutic benefits while reducing the risk of adverse reactions [39],[40].

1.4.3. Chronotherapy

Chronotherapy adjusts the timing of doses to the body's biological cycles and behavioural tendencies in order to maximise any positive effects and/or minimise any negative effects

throughout the day and night when taking medicine [41]. A medication's pharmacokinetics and pharmacodynamics responses to circadian rhythms can have a major impact on a drug's effectiveness and/or toxicity [19].

1.5. PHARMACOKINETIC MODELLING

Pharmacokinetic (PK) and pharmacodynamic (PD) modelling can be a helpful tool to investigate the impact of circadian rhythms and chronotherapy on the treatment of hypertension and rheumatoid arthritis. Pharmacokinetic/Pharmacodynamic (PK/PD) models connect medication concentration with therapeutic impact and can be used to identify the optimal dosing time to increase effectiveness and reduce side-effects [42],[43].

Physiologically based pharmacokinetic (PBPK) models start from knowledge of the body's physiology (the system) and of a drug's physicochemical characteristics to predict plasma concentration-time profiles. Population pharmacokinetic (popPK) models account for individual differences in drug exposure and can inform on the need for dose adaptation in subsets of patients. Population pharmacokinetic-pharmacodynamic (pop-PKPD) models integrate both PK and PD variability in a group of patients, making them useful for investigating the impact of circadian rhythms and chronotherapy on hypertension and rheumatoid arthritis treatment. These models can clarify how chronotherapy affects drug responsiveness by pinpointing the variables that affect drug response. However, since limited literature is currently available on this topic, it may be necessary to employ a combination of various models to fully understand the impact of chronotherapy on medication response.

Circadian rhythms may be described mathematically using the Population-Phase-Model-of-Oscillator-interactions (P-PPK) model. This mathematical model explains how several circadian oscillators, which oversee creating and maintaining the circadian rhythm, interact with one another. The oscillators and their interactions combined with the impacts on numerous behavioural variables including light, temperature and genetic control are accounted for [44]. The Goodwin model, which depicts the interactions between the genetic

feedback loops that control the development of the circadian rhythm, is one variation of the P-PPK model [45]. The suprachiasmatic nucleus, the main pacemaker of the circadian rhythm, has many oscillators that interact with one another. The Kronauer model, another variation of the P-PPK model, depicts these relationships. With the use of these models, researchers may examine a variety of circadian phenomena, including entrainment to light-dark cycles, phase changes in response to outside cues and the impact of genetic mutations on the circadian rhythm [46].

24-Hour oscillations in blood pressure (BP) levels are a hallmark of circadian rhythms of BP. To represent the temporal course of BP in the absence of medication, current PKPD models for circadian BP oscillations use several harmonics, often two cosine functions. The difference between the observed BP profile with and without the drug is then used to evaluate the drug's impact. Even though these models accurately predict the circadian rhythm for the typical patient, they frequently lack between-subject variability for the cosine function's amplitude and phase shift. Furthermore, these variables may not accurately reflect the physiological mechanisms that underlie the circadian regulation of BP. As a result, it is difficult to estimate the impact of antihypertensive medication for individuals with changing physical activity or day/night cycles. Therefore, the above-mentioned mechanism-based models, such as a PKPD model, are to be preferred [47].

2. OBJECTIVES

The main objective of the master thesis is the investigation of the pathogenesis of diseases with established oscillatory rhythms and the exploration of chronotherapy as a potential treatment approach for hypertension and rheumatoid arthritis. The thesis will also aim to examine the influence of circadian rhythms on the pharmacokinetics and pharmacodynamics of medication to achieve maximal drug concentrations at the intended site of action within the body at the right moment, to maximize efficacy and limit side effects.

Specifically, the thesis will:

1. Present a thorough evaluation of the literature on the pathogenesis of diseases with established oscillatory rhythms, in particular hypertension and rheumatoid arthritis.
2. Explore the mechanisms by which chronotherapy can be used to prevent or improve symptoms of these diseases.
3. Investigate the influence of circadian rhythms on the pharmacokinetics and pharmacodynamics of medication.
4. Investigate the use of PK/PD models to explore the influence of chronotherapy on rheumatoid arthritis and hypertension.
5. Identify areas for future research and potential clinical applications of chronotherapy in the treatment of these and other diseases.

With a focus on the use of chronotherapy to optimize drug concentrations and enhance treatment outcomes, this master's thesis seeks to provide an in-depth understanding of the connection between oscillatory rhythms, circadian rhythms and the treatment of hypertension and rheumatoid arthritis.

Different literature websites like PubMed and Web of Science were used with the intention to find an answer to the previous mentioned research questions. This will be further explained in Materials and Methods.

3. MATERIAL AND METHODS

3.1. METHODS USED FOR INTRODUCTION

A literature search was carried out to look for background related to the research topics of this master thesis. A thorough review of the literature was done to find articles that concentrated on the chronotherapeutic elements of drug therapy. The search was performed in 2023 using the terms "Circadian rhythms", "Chronopharmacology", "Chronopharmacokinetics", "Chronopharmacodynamics", "Chronoefficacy", "Chronopathology", "Chronotoxicity", "CLOCK-BMAL1 genes", "The circadian clocks", "Shift workers", "Jetlag", "Circadian rhythm disruption". PubMed and Web of Science databases were used for the search. Publications were limited to the English language, year (2000-) and accessible articles. Further, duplicate articles were removed. Then, paper abstracts were read and sorted by relevance. After selection for review, selected articles were re-read again to classify them based on the operational definitions (Figure 3.1.).

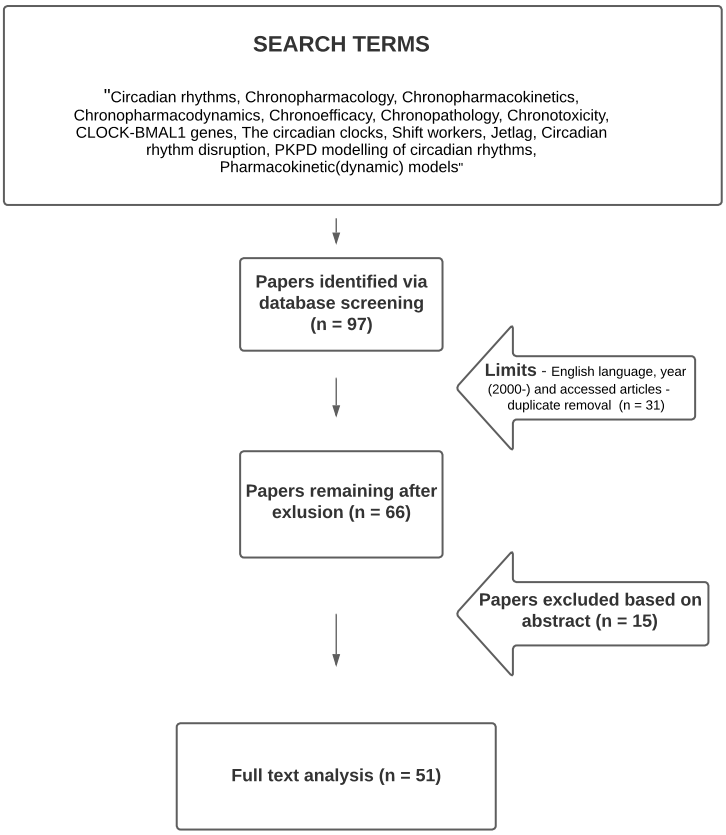

Figure 3.1. Study screening flowchart for the introduction. Created using Lucidchart.

3.2. METHODS USED FOR RESULTS

To address the research topics of this master thesis, an in-depth literature search was conducted. The focus was finding articles related to the chronotherapy of hypertension and rheumatoid arthritis. To locate relevant articles, a thorough review of the literature was performed using various search terms such as "Pharmacodynamics hypertension", "Antihypertensive effect", "Blood pressure lowering" OR "Blood pressure reduction", "Chronotherapy hypertension", "Chronopharmacokinetic hypertension", “Chronopharmacology rheumatoid arthritis”, “Pharmacodynamics rheumatoid arthritis”, “Chronotherapy rheumatoid arthritis”, “Pharmacokinetics rheumatoid arthritis”, ”PKPD rheumatoid arthritis”, “Chronotherapy glucocorticoids” in databases like PubMed and Web of Science. Only articles published in English from 2000 onwards were considered. Additionally, the reference list of the retrieved articles was reviewed to ensure that no publications were missed. Duplicate articles were removed, and the remaining paper abstracts were assessed for relevance. After selection for review, the articles were re-read and classified based on operational definitions (Figure 3.2.).

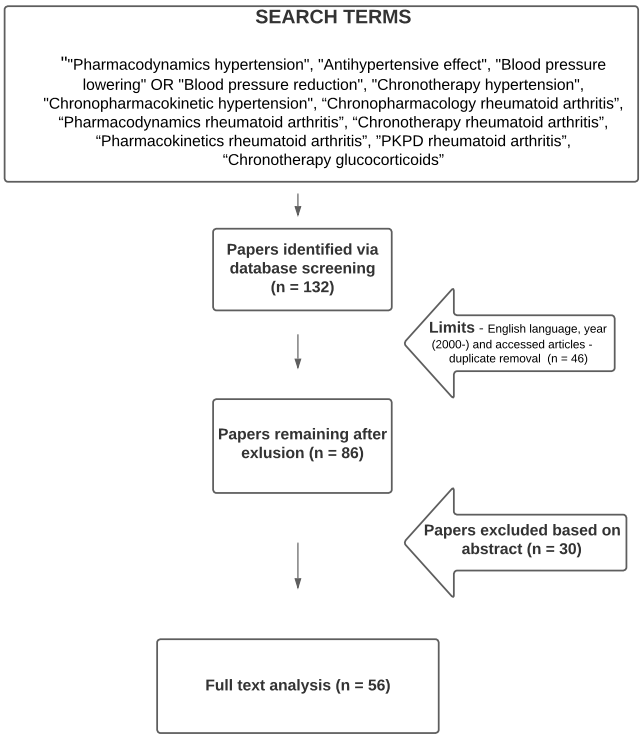

Figure 3.2. Study screening flowchart for the results. Created using Lucidchart.

3.3. METHODS USED FOR PKPD-MODELLING

A thorough review of the literature was performed using various search terms such as "Circadian models", "Circadian PKPD models", "Poppk models AND/OR circadian parameters", "pharmacometrics models", "PBPK models for hypertension OR RA", "PKPD models for hypertension OR RA", "Monolix for hypertension OR RA", "P-PPK-models", "P-PPK-models for hypertension OR RA", "Circ* models AND pkpd* AND hypertension", "Circ* AND pkpd* AND antihypertensive medication", "Circ* AND poppk* AND hypertension", "Circ* AND pbpk* AND hypertension" in databases like PubMed and Web of Science. Only articles published in English from 2000 onwards were considered. Duplicate articles were removed, and the remaining paper abstracts were assessed for relevance. After selection for review, the articles were re-read and classified based on operational definitions (Figure 3.3.).

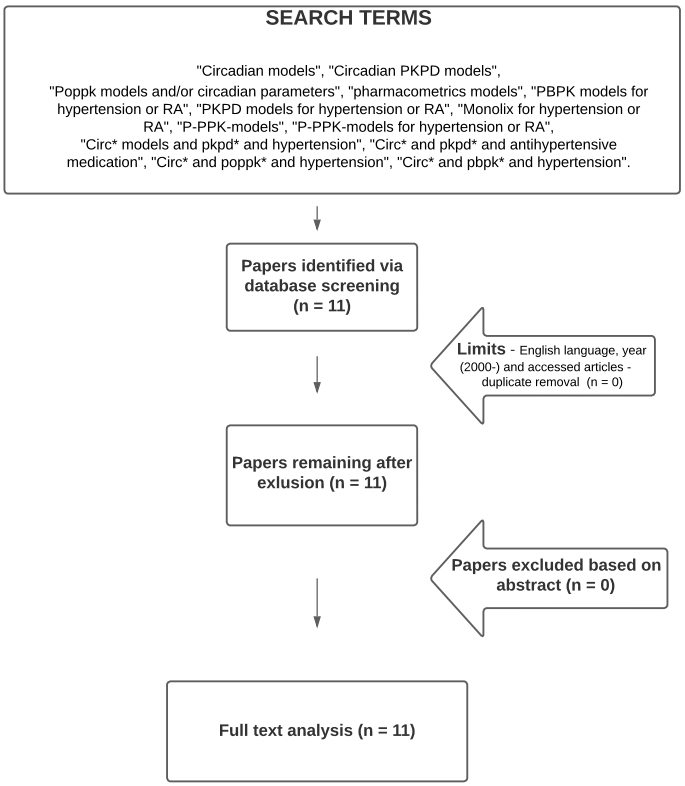

Figure 3.3. Study screening flowchart for the results. Created using Lucidchart.

In this thesis, the linear two-compartmental PK/PD model is used to analyse the concentration-time profiles of free prednisone and free prednisolone. Each compound's plasma concentration-time profiles followed a one-compartmental PK. Prednisone is easily converted to prednisolone and back again (grey area in Figure 3.4). To describe the concentration-time profiles, measurements of the free concentrations of prednisone and prednisolone (C_{free}^{PN} and C_{free}^{PNL}) in each compartment were made. The compounds' conversion clearances ($CL^{PNL-->PN}$ and $CL^{PN-->PNL}$) and elimination clearances (CL^{PNL} and CL^{PN}) as well as their volumes of distribution (V^{PNL} and V^{PN}), were calculated. After being taken orally, prednisone was thought to be absorbed according to first-order kinetics.

By studying the natural circadian rhythm of cortisol and its suppression by prednisolone, one can gain insights into the timing of peak and trough cortisol levels. This knowledge can help in synchronizing the administration of prednisone and prednisolone to coincide with the body's own cortisol fluctuations. A linear release model with an inhibition effect of prednisolone was used to describe the cortisol release rate (R^{COR}). In this model, the first-order elimination rate constant (k_e^{COR}) was incorporated to represent the rate of cortisol elimination from the system (Figure 3.4.). The systemic activities of prednisone and prednisolone were assessed by calculating cumulative cortisol suppression (CCS%) during 24 hours. This was done by comparing the area under the cortisol baseline curve ($AUC_{baseline}^{COR}$) with the area under the curve of plasma cortisol concentrations after prednisolone exposure ($AUC_{treatment}^{COR}$). The trapezoidal rule was applied to calculate the area under the curve in both cases.

Examining the impact of prednisolone on lymphocyte count allows to assess whether the timing and dosing of prednisone/prednisolone administration influence the natural circadian variations in lymphocyte trafficking, which is part of the immune system's circadian rhythms. By studying this relationship, one can gain insights into how prednisone/prednisolone affect the rhythmic fluctuations in lymphocyte trafficking and can determine if optimizing the timing of prednisone/prednisolone administration aligns with the body's natural immune system patterns. The total lymphocyte trafficking was described by a first-order elimination process (k_{out}^{LYM}) and a zero-order production process (k_{in}^{LYM}) with

inhibition effects from both endogenous cortisol and exogenous prednisone/prednisolone (right part, Figure 3.4.). Similar to CCS, altered total lymphocyte trafficking during 24 hours was evaluated by comparing the area under the effect curve without prednisone/prednisolone ($AUC_{baseline}^{LYM}$) with the area under the curve after prednisone/prednisolone exposure ($AUC_{treatment}^{LYM}$). The trapezoidal rule was used to calculate the area under curve.

The aim is to achieve a lower lymphocyte count, as it corresponds to a reduction in the immune-mediated inflammatory processes which helps alleviate the symptoms of the disease, as well as reduce the levels of cortisol through the administration of prednisone/prednisolone, leading to decreased inflammation and improved symptom management.

In order to study prednisone and prednisolone PK's dependence on administration day/night time and to expand the PK to the effects of dosing time on PD, an interactive algorithm based on the PKPD model presented in article [48] was applied. Two interactive Microsoft Excel spreadsheets were used to simulate the effects of various intravenous prednisolone or oral prednisone dosages and administration periods (the interface of the spreadsheet is shown in Figure 8.1. and 8.2. in Appendix I). Based on input data such as the drug name, dosage quantity, administration time, dosing regimen and dosing interval (τ), the spreadsheets allow real-time prediction of PK and PD profiles, anticipated cumulative cortisol suppression (CCS%) and area under the effect curve (AUEC%). CCS% measures cumulative therapeutic success, while the AUEC% quantifies overall efficacy by calculating the area under the efficacy curve.

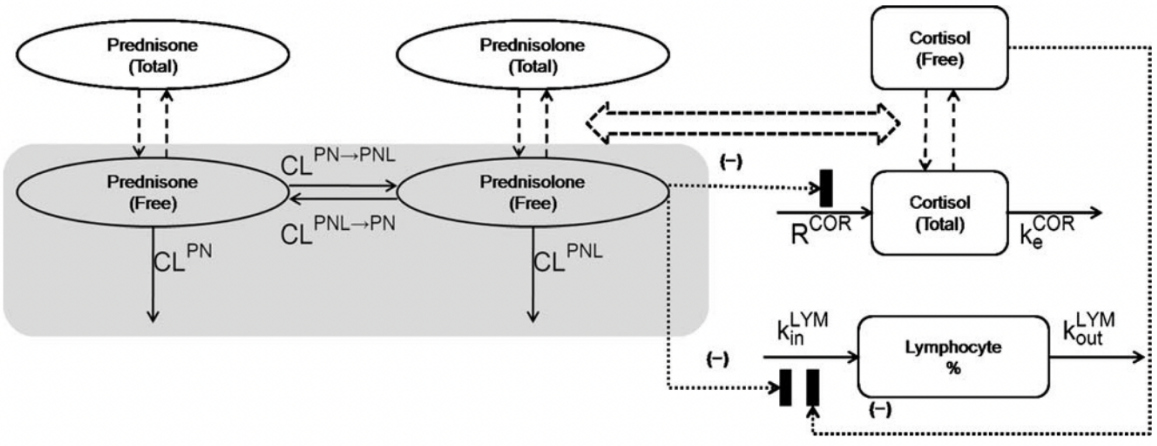

Figure 3.4. PKPD model of prednisone and prednisolone. CL^{PNL}: prednisolone clearance, CL^{PN}: prednisone clearance. $CL^{PNL-->PN}$ and $CL^{PN-->PNL}$: conversion clearances between free prednisolone and prednisone. R^{COR}: cortisol release rate, k_e^{COR}: cortisol elimination rate constant. k_{in}^{LYM}: lymphocyte production rate constant, k_{out}^{LYM}: lymphocyte elimination rate constant. (-): inhibitory effect of prednisolone [48].

Prednisone, the precursor form of prednisolone, is clinically administered orally in doses ranging from 5 to 60 mg. In the Excel spreadsheet, a simulation was conducted involving doses ranging from 0 to 60 mg, with 20 mg intervals. The simulation spanned a 24-hour clock time period with hourly intervals, for a multiple oral dose regimen with an interval of 24 hours (Table 3.1.), (Figure 8.1. in Appendix I). CCS% (Figure 8.3. in Appendix I), AUEC% (Figure 8.4. in Appendix I) and C_{trough}, which indicates the lowest prednisolone concentration before the next dose (Figure 8.5. in Appendix I), were used to evaluate prednisone's effectiveness and pharmacokinetics at steady state.

For bolus IV administration, prednisolone doses ranged from 0 to 100 mg, with 20 mg intervals, over a 24-hour period with hourly intervals were simulated. The simulation spanned a 24-hour clock time period with hourly intervals, for a multiple IV bolus doses administered with an interval of 24 hours (Table 3.1.), (Figure 8.2. in appendix I). CCS% (Figure 8.6. in Appendix I), AUEC% (Figure 8.7. in Appendix I) and initial prednisolone concentrations (C_0) (Figure 8.8. in Appendix I) are used to evaluate prednisolone's effectiveness and pharmacokinetics.

Contour plots extrapolate these variables to show how they relate to one another, which helps in dosing optimization, understanding dose-response, and assessing efficacy and pharmacokinetics.

Table 3.1. the simulation scenario for oral and IV bolus administration in the Excel spreadsheet.

	Oral administration	IV administration
Drug name	Prednisone	Prednisolone
Dose (mg)	0 - 60	0 - 100
Time of administration (h)	0 - 24	0 - 24
Dose regime	Multiple	Multiple
Tau (dose interval, h)	24	24

4. RESULTS

4.1. HYPERTENSION

The medical disease known as hypertension is defined by an increased BP in the arteries. The force that the blood applies to the artery walls as it flows through them is known as BP. A blood pressure reading is often expressed as two numbers: the systolic blood pressure (SBP), which is the top number, and the diastolic blood pressure (DBP), which is the bottom number and which represents the pressure while the heart is at rest in between beats. Normal blood pressure is often thought to be about 120/80 mmHg. When the blood pressure is continuously at or above 140/90 mmHg, hypertension is identified. A blood pressure reading greater than 110/65 mmHg during sleep time is considered night-time hypertension [49]. When three antihypertensive medications are continuously taken at the recommended dosage while lifestyle adjustments are made, and the blood pressure still rises above 140/90 mmHg, it is considered resistant hypertension. Resistant hypertension is high blood pressure that fails to respond to therapy with various drugs. Pseudo resistant hypertension is a lack of BP regulation resulting from incorrect measurement, non-compliance, poor medication selection/dosage or the white coat effect. An elevated BP without evidence of target organ harm is what the "white coat effect" is known as [51].

Hypertension is a significant risk factor for several serious health conditions, including heart disease, kidney disease, stroke and vision loss. Also, it can cause damage to blood vessels throughout the body, leading to an elevated risk of aneurysms, peripheral artery disease and other complications [41]. It can often be managed through lifestyle changes, such as physical activity, diet and medication if necessary. It's important to monitor and manage hypertension to lower the risk of complications and elevate overall health outcomes [52].
It has long been believed that blood pressure stays constant throughout the day. Recent studies, however, have demonstrated that blood pressure varies according to a circadian rhythm, with greater values during the day and lower levels at night. Blood pressure readings taken just when awake in the doctor's office might not be representative of its level at other times of the day. To obtain a more accurate picture of blood pressure

throughout the day and spot any unusual pattern or swing, it is advised to take blood pressure readings at various times, while engaged in various activities, and in various postures [53],[54]. The increasing use of round-the-clock ambulatory blood pressure monitoring (ABPM) technologies to conduct BP investigations and identify hypertension demonstrates that systolic blood pressure and diastolic blood pressure change as part of a predictable-in-time 24-hour pattern of generally higher awake-time and lower asleep-time values [55],[56].

4.1.1. Chronotherapy in hypertension

The well-known circadian rhythm of arterial blood pressure, that shows a 24-hour pattern and is characterized by a morning rise and nocturnal decrease, is connected to the increase in unfavourable cardiovascular events during the early morning period, for example acute myocardial infarct (Figure 4.1.) [53]. The elevation in unfavourable cardiovascular events can be attributed to physiological changes that occur in the morning, including an increase in BP, coagulation, heart rate and vascular tone, as well as a decrease in fibrinolysis and coronary flow. Similar circadian timing patterns for sudden cardiac death, ventricular arrhythmias, stroke and arterial embolism have been shown [41],[57].

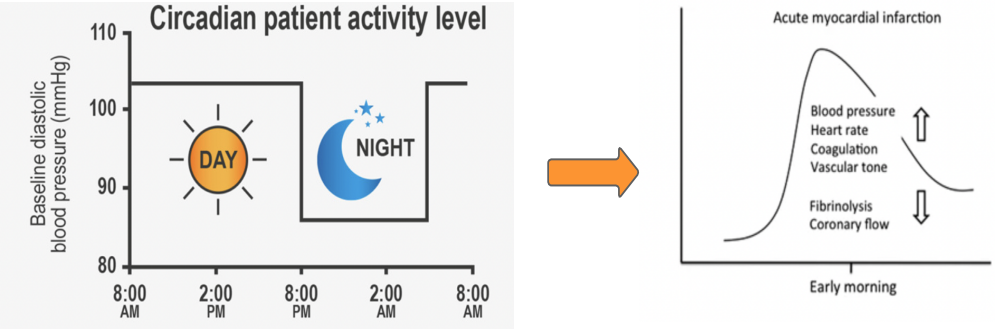

Figure 4.1. The circadian rhythm of arterial blood pressure. Created by Lucidchart.

The kidneys play a crucial role in regulating BP, and their ability to excrete sodium is thought to be a key factor in the overnight drop in blood pressure. This decrease in BP during sleep is partially attributed to the kidneys' capacity to eliminate sodium from the

body, leading to a reduction in fluid volume and consequently lowering blood pressure. However, when renal function is impaired (for example because of chronic kidney disease (CKD), kidney stones or infection), sodium excretion during the day is reduced, leading to fluid retention and higher blood pressure. To compensate, the body triggers pressure natriuresis mechanisms at night to increase sodium output, but this can cause a rise in blood pressure during sleep. In conclusion, this night-time hypertension can be attributed to the body's attempt to correct the sodium imbalance caused by impaired renal function during the day [53]. Reduced baroreceptor sensitivity, poor vascular compliance and increased sympathetic tone are additional variables that lead to higher nocturnal blood pressure [59].

Conventionally, healthcare professionals advise hypertensive individuals to take their blood pressure-lowering medication in the morning when they start their daily activities. This recommendation is based on large epidemiological studies that have found a higher incidence of cardiovascular events such as sudden cardiac death, haemorrhagic stroke, myocardial infarction, angina pectoris and ischemic stroke in the morning. As stated before, the morning surge in blood pressure, commonly known as the "morning peak," is believed to be one of the contributing factors to these cardiovascular events. During the early morning hours, there is a natural increase in sympathetic nervous system activity and hormonal fluctuations that can lead to elevated blood pressure levels. This is especially relevant for individuals with hypertension, as their blood pressure may already be higher than normal. To counteract this morning surge in blood pressure and reduce the risk of cardiovascular events, healthcare professionals recommend taking blood pressure-lowering medication in the morning [54]. However, there is an alternative approach known as chronotherapy that suggests taking blood pressure-lowering medication in the evening instead. Chronotherapy considers the natural variations in BP throughout the day and aims to align medication timing with these fluctuations. The rationale behind evening dosing in chronotherapy is based on the understanding that blood pressure follows a circadian rhythm, with lower levels during sleep and higher levels upon waking. By taking medication in the evening, it is believed to have a greater effect than the conventional therapy during

the early morning hours, thereby counteracting the morning surge in blood pressure and potentially reducing the risk of cardiovascular events (Figure 4.2.) [60].

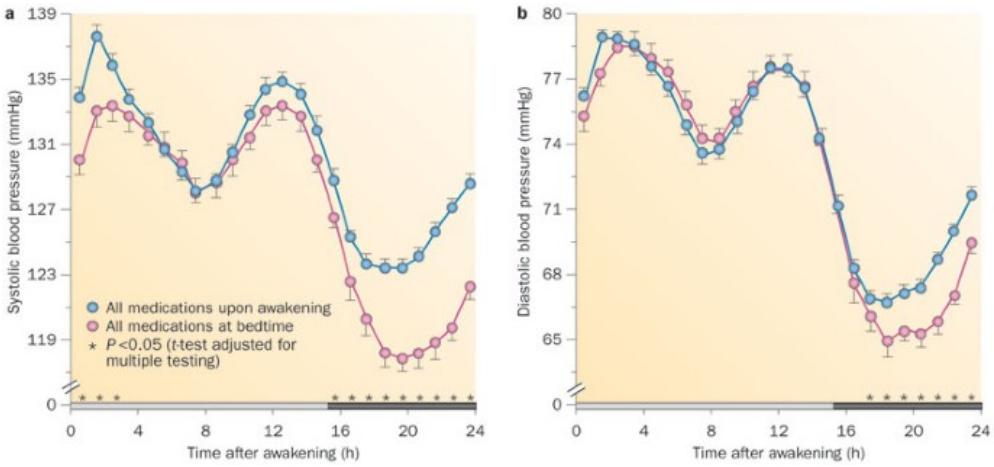

Figure 4.2 Representation of the SBP and DBP after taking antihypertensive medication upon awakening vs bedtime [60].

This strategy faces challenges because antihypertensive drugs have a short duration of action, which can result in suboptimal drug concentrations the following morning, falling below the minimum effective concentration (MEC). Current treatments for hypertension are developed to deliver medications at a constant rate to establish a smooth and stable drug concentration within the therapeutic range during the 24-hour dosing period. To address this problem, several technologies, including extended-release formulations, delayed-release formulations and chronotherapeutic drug delivery systems, have been created. (Figure 4.3.) [61],[54]. These innovations attempt to adequately manage morning BP by keeping medication concentrations at therapeutic levels throughout the night and into the morning. Extended-release formulations are made to release the medication gradually over an extended period, keeping the systemic drug concentration constant. Delayed release is a form of drug delivery system in chronotherapy that aims to time the release of medication to align with the peak of the morning increase in blood pressure. Other examples of tablets and capsule-based delivery systems of varying complexity, are:

1. A biodegradable geometric system made up of two slowly hydrating barriers and a hydrophilic matrix core that degrades gradually to allow medicine to diffuse steadily as it passes through the gastrointestinal tract system (GITS).
2. Core coat system, which surrounds the active ingredient with a hydrophilic gel layer to allow for constant drug diffusion while the dosage form travels through the GITS.
3. A polysaccharide sodium alginate system that turns gelatinous as it absorbs water in the GITS.
4. To achieve constant drug dispersion, encapsulated beads with coatings of varying thickness or polymer composition may be used.

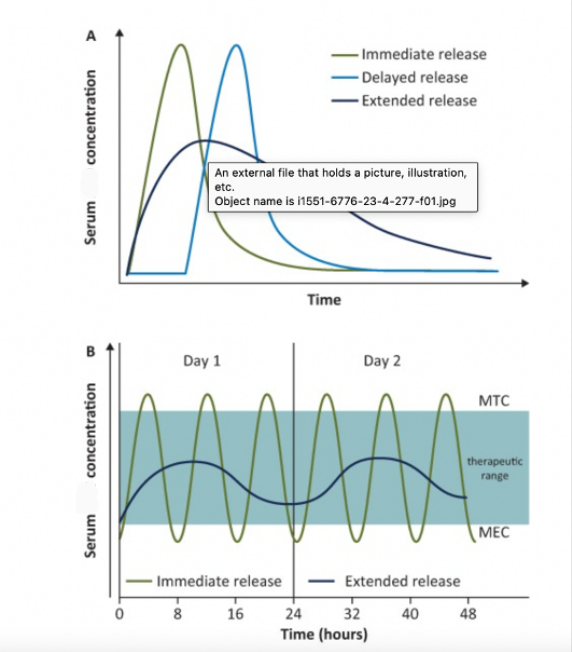

Figure 4.3. Schematic representations of variations in the serum drug concentration (SDC) for various drug administration methods over time. MTC and MEC stand for minimal toxic concentration and minimal effective concentration, respectively [61].

So, chronotherapy offers several benefits in managing BP, reducing the risk of CVD and preventing organ damage to the kidneys, brain, eyes and heart. It allows for individualized medication based on the patient's circadian cycle [52],[57]. Specifically, in hypertensive patients, chronotherapy targets the elevated morning BP, aiming to normalize daytime, nocturnal and 24-hour BP levels and establish a typical blood pressure pattern. This helps

lower the likelihood of coronary disease [62],[41]. Furthermore, taking antihypertensive medication at night has been associated with improved renal function, decreased levels of low-density lipoprotein (LDL) cholesterol and increased levels of high-density lipoprotein (HDL) cholesterol. Night-time administration of antihypertensive medication also reduces the risk of diabetes and CKD. Specifically, bedtime treatment has been found to effectively lower blood pressure among individuals with CKD (Figure 4.4.) [57].

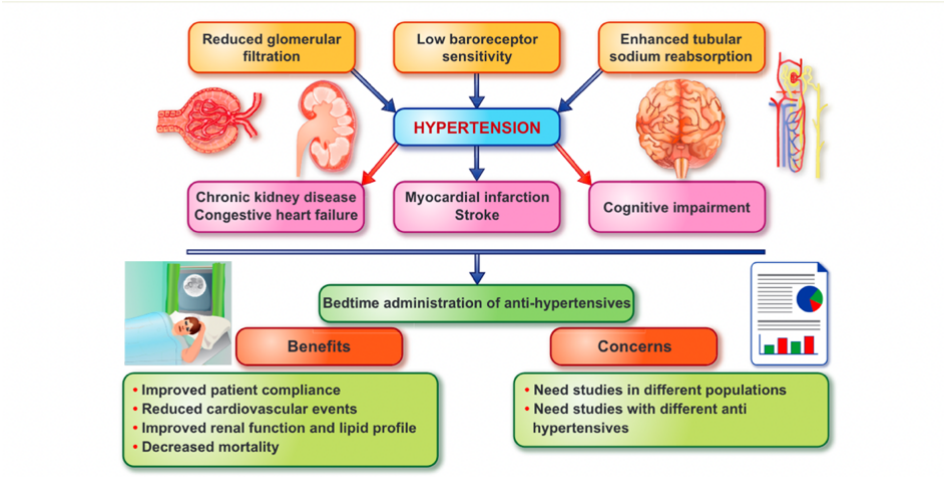

Figure 4.4. Hypertension and chronotherapy: pathogenesis, advantages, and drawbacks [57].

4.1.2. Antihypertensive medication

The main groups of antihypertensive drugs, such as calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI), alpha- and beta-adrenoceptor blockers and angiotensin II receptor blockers (ARB) typically show stronger BP-lowering effects when taken in the evening compared to the morning. In addition, aspirin may also be used as an hypertensive medication [41]. Angiotensin II is a hormone that is essential for controlling the body's electrolyte balance and blood pressure. It is created by the complex combination of hormones and enzymes known as the renin-angiotensin-aldosterone system (RAAS), which controls blood pressure. ARBs and ACEIs lower the angiotensin II levels in the body. ARBs function by inhibiting angiotensin II from attaching to and activating the receptors at their target locations in the body. They reduce blood vessel constriction, which lowers blood pressure. ACEIs prevent the enzyme from turning angiotensin I into angiotensin II. As a result, the body produces less angiotensin II, which in turn lowers blood pressure and reduces fluid retention.

4.1.2.1. Calcium channel blockers (CCB)

The impacts of evening versus morning CCB administration are reviewed, including amlodipine, cilnidipine, diltiazem, isradipine, nifedipine, nisoldepine, nitrendipine and verapamil. When Verapamil is taken before bedtime, it exhibits a half-life kinetics that results in a decrease in its level during the evening and overnight hours. This leads to lower concentration during deep sleep when blood pressure is typically at its lowest. As a result of verapamil's decreased concentration during deep sleep, the drug's desired MEC may not be achieved the following morning [63]. Therefore, verapamil with a controlled-onset extended-release profile (COER) is developed. This was the first unique drug-delivery tablet created with chronotherapy of hypertension in mind. Verapamil's suggested night-time consumption is delayed for 3-4 hours by the tablet's drug delivery system (Figure 4.5.) [64]. Therefore, thanks to the drug delivery technology of this tablet, the highest blood concentration is reached in the morning around the time of wakening and an increased level is maintained throughout daily activity [52].

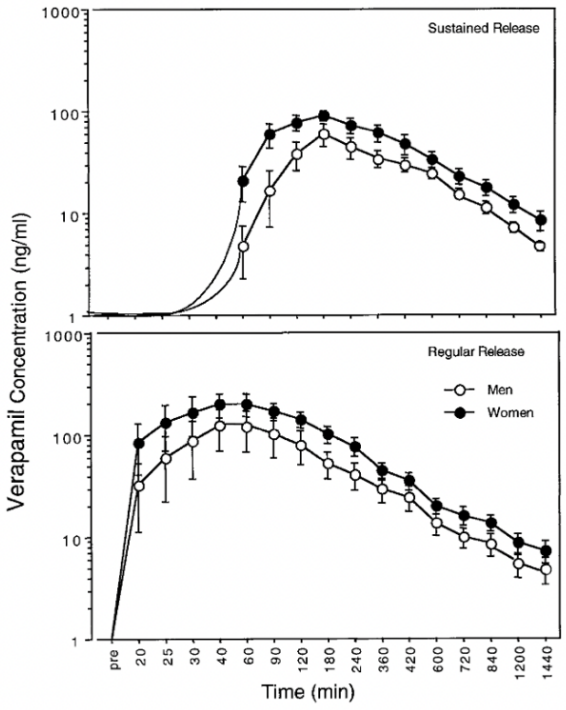

Figure 4.5. Controlled onset extended release plasma profiles vs regular release plasma profiles of verapamil in men and women [64].

Another special delivery form is the GITS formulation of nifedipine that keeps the drug concentration almost constant throughout the course of a 24-hour period.

Graded release long-acting diltiazem ingestion at 10 p.m. results in the desired PK profile for chronotherapy of hypertension (Figure 4.6.) [66]. Due to the delayed and gradual drug release after dosage, the blood diltiazem concentration is at its lowest while one is sleeping at night and at its highest in the morning [67],[68]. On the contrary, when taken a dosage at 6 a.m., the lowest drug level develops in the morning and the highest drug level develops in the evening. The discrepancy between the highest and lowest diltiazem blood concentrations in relation to the circadian pattern of BP hypertension highlights how crucial it is for patients and doctors to adhere to the recommended dosage time for this and other unique bedtime-formulated BP chronotherapies [52],[69].

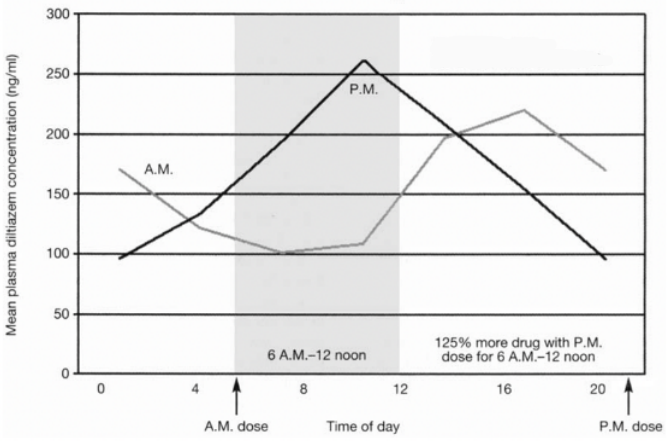

Figure 4.6. Mean plasma concentrations of graded release long-acting diltiazem [66].

The efficacy of dihydropyridine derivatives in decreasing blood pressure is dependent on its PK. Dihydropyridine derivatives with various PK characteristics include amlodipine, cilnidipine, isradipine, nifedipine, nitrendipine and nisoldepine. Due to amlodipine's prolonged half-life (30-50h) and gradual beginning of effect, a single dosage can continuously reduce blood pressure for up to 24 hours, therefore there is no need for special delivery forms [41],[53]. The same phenome is seen with cilnidipine and isradipine that can offer 24-hour blood pressure management, with a once-daily dose schedule, due

to their intermediate pharmacokinetic features (drugs that have moderate rates of ADME). Although nitrendipine (12-24h) and nisoldepine (6-9h) also have extended half-lives, they are unable to lower blood pressure quickly due to their late onset of action [70]. In addition, the frequency of developing peripheral oedema, a disadvantage of using dihydropyridine derivatives, can be decreased with bedtime dosing [71].

4.1.2.2. Angiotensin-converting enzyme inhibitors (ACEI)

Benazepril, quinapril, ramipril, enalapril, perindopril, spirapril and trandolapril are just a few of the ACEIs whose effects are compared between early and late administration. In every instance, taking these drugs in the evening have more noticeable impact on blood pressure and significantly alter the circadian blood pressure profile [72]. When comparing diltiazem (CCB) to ramipril, it is shown that diltiazem is more effective reducing early morning BP, morning heart rate and the heart rate-pressure product (Figure 4.7.). This is because diltiazem, when administered at bedtime, follows the circadian rhythm of BP and heart rate, making them more effective at reducing early morning hemodynamic effects. While ramipril can be more effective in reducing blood pressure when taken in the morning due to circadian variation of hepatic esterase activity, giving ramipril in the evening may result in reduced conversion to its active form, leading to decreased efficacy at reducing night-time blood pressure [73],[74].

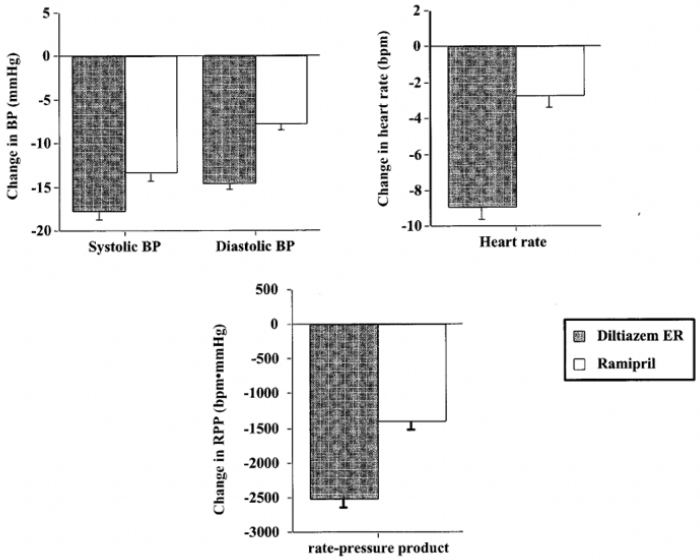

Figure 4.7. Diltiazem vs ramipril [70].

The effectiveness of spirapril, a medication with a half-life of 40 hours, appears to be influenced by the timing of administration. Morning dosing of spirapril has been found to have slightly higher efficacy compared to bedtime dosing when considering its overall effects. However, when specifically targeting the reduction of mean diurnal BP to reduce nocturnal BP and reduce the risk of early morning CV events, bedtime administration of spirapril has been observed to be more successful than morning administration [75].

4.1.2.3. α-adrenoceptor antagonists

Peripheral resistance is the term used to describe the resistance that blood faces when it passes through blood arteries. The smooth muscles in blood vessel walls, which are influenced by several neurotransmitters and hormones, are principally responsible for controlling this resistance. The sympathetic nervous system, which produces norepinephrine that acts on α-adrenoceptors on the smooth muscle cells of blood arteries to cause them to contract and raise resistance, is a key regulator of peripheral resistance. In comparison to other times of the day and night, α-adrenoceptor inhibition reduces peripheral resistance more effectively in the early morning. Doxazosin, an α-blocker, has a significantly long half-life, which indicates that it is active in the body for a considerable amount of time. Doxazosin can offer persistent α-adrenoceptor inhibition when taken at night, which may help explain why it has a greater effect in the morning [76].

4.1.2.4. β-adrenoceptor antagonists

After taking the β-antagonist propranolol in the evening, there is a decreasing blood concentration in the morning (caused by the deliberate delaying of propranolol release for 4-5 hours) with a peak level between 4 and 10 a.m. Throughout the entire 24-hour dosing time, there is significant persistence of the BP-lowering effect [77]. Regarding nebivolol and hypertension treatment, some studies have indicated that taking nebivolol in the evening is more beneficial in terms of reducing the diurnal/nocturnal blood pressure ratio. By taking nebivolol in the evening, when blood pressure is normally lower, the medication

may better align with the natural rhythm and help to lower blood pressure during the night and early morning [68].

4.1.2.5. Angiotensin II receptor blockers (ARB)

Due to their effectiveness and tolerability, ARBs are increasingly becoming a popular choice for treating hypertension. Similar to ACEIs, these drugs exhibit differences in therapeutic effects depending on the time of intake, regardless of the medication's half-life [54]. A valsartan dose taken at night causes a higher decrease in the ratio of diurnal to nocturnal blood pressure [78]. Additionally, giving valsartan, olmesartan or candesartan at bedtime causes a significant decrease in urinary albumin excretion, which is strongly correlated with the reduction in mean asleep systolic blood pressure and the expansion of sleep duration in relation to the decline in systolic blood pressure [79]. The ARB telmisartan, which has an extended half-life, provides additional advantages when taken at night, such as improved sleep quality and a reduction in blood pressure. Similar conclusions apply to olmesartan [41]. Fimasartan is a freshly created kind of ARB that targets this receptor specifically. Its substantial enterohepatic recirculation greatly lengthens its half-life and increases drug exposure [44].

Compared to other ARBs, azilsartan has a higher affinity for the receptor AT1 receptor than candesartan. The medication is anticipated to have a stronger and longer-lasting impact on blood pressure [80]. Azilsartan lowers overnight SBP more effectively than candesartan. This may be caused by azilsartan's ability to decrease sodium reabsorption, strong effects on the renin-angiotensin-aldosterone system, abnormal sympathetic nervous system activity, improved glucose tolerance and insulin sensitivity [53]. The evening use of candesartan decreases blood pressure during the night while simultaneously lowering left ventricular hypertrophy and microalbuminuria [62].

4.1.2.6. Other antihypertension medication

Torasemide, a loop diuretic, works better when taken before bed than when taken after waking up. The time-response curves only showed complete 24-hour therapeutic coverage

when the medicine was administered at night. When taken in the morning, the duration of activity was just 15h. Additionally, the diurnal/nocturnal BP ratio is only decreased by torasemide intake at night [81],[82].

Historically, a low dose of aspirin (100 mg/day) has been used as an anticoagulant to avoid cardiovascular events. 24-hour mean SBP and DBP were slightly elevated after taking a morning modest dosage (100 mg/day) of aspirin. In contrast, taking low-dose aspirin at night greatly lowers blood pressure. The correlation between night-time administration and decreased morning platelet reactivity suggests that aspirin taken at night has limited use for lowering blood pressure in people with advanced hypertension, but is helpful in lowering cardiovascular risk in the morning [83]. It can therefore be used for mild hypertension [84]. These results are due to the diurnal inhibition of angiotensin II by aspirin [41]. When taken daily at the appropriate biological time (in the evening), a modest dose of aspirin not only represents a good strategy for the secondary prevention of cardiovascular risk and illness, but also for BP management in moderate hypertension [85].

4.1.2.7. Clinical outcomes of chronotherapy (PD)

It's critical to assess if taking blood pressure drugs at night improves clinical outcomes for hypertension regardless of blood pressure fluctuations. For instance, switching the timing of antihypertensive drug delivery from the morning to the evening was linked to a decrease in proteinuria in CKD patients [86]. When compared to placebo, using ramipril was linked to a reduction in the incidence or severity of cardiovascular outcomes [53]. A considerable reduction in overall CVD morbidity, death risk and an improvement of renal function is associated with dosing at night [57]. Death from any cause, myocardial infarction, haemorrhagic stroke, coronary revascularization, ischemic stroke, rupture of aortic aneurysms, heart failure, thrombotic occlusion of the retinal artery, angina pectoris, acute arterial occlusion of the lower extremities and transient ischemic attack are among the reasons that fall under this category [53]. As a result, patients on the bedtime-treatment regimen have lower heart rates for the vital CVD outcome than patients who take all of their drugs when they wake up [62].

Studies have reported significant benefits associated with administering hypertension treatment at bedtime or in the evening, including improved reduction in asleep systolic blood pressure, decreased occurrence of adverse effects, reduced albuminuria, decreased left ventricular mass, increased relative decline in sleep-time systolic blood pressure, increased glomerular filtration rate and decreased left ventricular posterior diameter [54].

4.2. REUMATOID ARTHRITIS (RA)

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the body's joints and other organs. The immune system attacking the lining of the joints causes inflammation, discomfort and stiffness, which is the underlying cause of the disorder. RA symptoms include joint pain, stiffness, limited range of motion, oedema, tiredness, weight loss and fever. One of the disease's most reliable diagnostic indicators is morning stiffness, which is present in virtually all patients. Multiple body systems, including the eyes, skin and lungs, can be affected by the signs and symptoms of RA, which can alter during the course of the day. The long-term effects of RA on joints include deterioration, deformity and disability [87],[88],[89]. Compared to a 24-hour cycle, the symptoms are at their worst in the morning.

The following are common drug treatments for RA:

1. NSAIDs: these drugs can relief pain and reduce inflammation. They are used as supporting therapy. Examples are aspirin, ibuprofen and naproxen.

2. Conventional synthetic disease-modifying antirheumatic drugs (DMARDs): they can slow down the progression of RA by suppressing the immune system. Examples are sulfasalazine (2 - 3 g/day), methotrexate (7.5 - 15 mg/week), leflunomide (10 - 20 mg/day) and hydroxychloroquine (200 - 400 mg/day).

3. Biologic DMARDs: these medications are a type of DMARD that are made from living cells and target specific proteins of the immune system. They are monoclonal

antibodies with specific interaction to specific cytokine or membrane markers. In case of RA typically combined with methotrexate. These medications are being used when conventional DMARD therapy fails. Examples include etanercept, infliximab, adalimumab and rituximab.

4. Targeted synthetic DMARDs: these medications have an impact on biological cascades that control cytokine production (Janus kinase inhibitor and phosphodiesterase 4 inhibition). In case of RA typically combined with methotrexate. These medications are being used when conventional DMARD therapy fails. Examples are tofacitinib, barcitinib and apremilast.

5. Corticosteroids: these medications can reduce inflammation and pain in the short term. Due to possible side effects, they are normally administered in small dosages for brief periods of time. They can be used as bridging therapy (15 – 30 mg/day) or maintenance therapy in combination with DMARD ($\leq$ 7.5 mg/day)

4.2.1. Circadian rhythms and RA

The immune system is one of several physiological processes that the human body's internal clock controls. Internal clock genes in immune cells let them control their activity during a 24-hour cycle. The immune system is therefore more active during the day and less active at night. Hormone production is assisted by the endocrine system, which also transmits timing signals from the SCN to the immune system and other parts of the body. While melatonin, acetylcholine and norepinephrine are produced more at night, cortisol, serotonin and dopamine are secreted more during the day (Figure 4.8) [89],[91]. Melatonin and prolactin are hormones involved in the immune response and energy regulation during sleep. They follow a 24-hour cycle, with their levels peaking at night. They play a significant role in increasing the release of cytokines, which are important signalling molecules for immune system function [88],[89]. Tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interferon gamma (IFN-γ) are examples of pro-inflammatory cytokines that are generated during sleep and boost immunity and resistance to infections [93]. Immune

system weakness brought on by circadian rhythm disruptions might result in possible illnesses like RA [75].

When there is a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, the body's ability to control inflammation may be negatively affected. This dysregulation can contribute to chronic inflammation in RA. One manifestation of this dysregulation is a decrease in night-time cortisol production in RA patients, which could explain why symptoms are more prominent in the morning (Figure 4.8.) [88],[89]. Cortisol, the primary hormone produced by the HPA axis, has potent anti-inflammatory properties. Reduced levels of cortisol at night may contribute to increased inflammation in RA. Additionally, there may be alterations in the circadian rhythm of melatonin synthesis in RA patients. It is important to note that while RA patients may have higher blood levels of IL-6 and these dysregulations in the HPA axis and melatonin synthesis, the circadian rhythms of cortisol, in terms of amplitude and length, may be similar between RA patients and healthy controls [92].

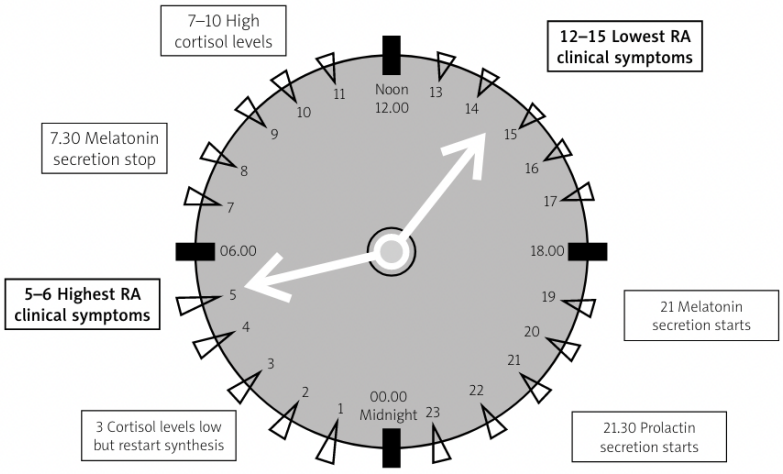

Figure 4.8. Clinical effects, such as morning symptoms like joint stiffness and pain, are determined by the circadian sequence of nocturnal hormone production, which activates and/or downregulates the immunological inflammatory response during the night [89].

Maintaining a healthy circadian rhythm is crucial for fostering ideal immune function and reducing the incidence of conditions like RA. Circadian rhythm issues may have a detrimental effect on the immune system and exacerbate RA symptoms. The immune

system uses a lot of energy during rest and circadian rhythm issues can interfere with how the body normally distributes energy, which exacerbates the immune system's problems [93].

4.2.2. Chronotherapy in rheumatoid arthritis

RA is induced by immune system attacks leading to joint injury and inflammation. By relaxing the immune system, glucocorticoids can aid in reducing the symptoms of RA. Relative adrenal insufficiency can occur in RA patients and exogenous glucocorticoids can operate as "replacement therapy" to increase the body's natural supply of these hormones [88],[94]. Low dosages of glucocorticoids are typically recommended when treating RA, particularly when used in conjunction with DMARDs that can delay the progression of RA and protect the joints from further damage [88],[95]. Glucocorticoids can be used either long-term to control chronic symptoms or short-term to manage symptoms until DMARDs take effect [89].

4.2.2.1. Glucocorticoids

In the treatment of RA, the timing of glucocorticoid administration is critical. Since disease-related activities occur in the morning, corticosteroids are frequently administered at night. For instance, it is thought that the greatest level of IL- 6 occurs during the early morning hours, when RA patients tend to suffer greater clinical symptoms. The immune system's cells and cytokine levels, such as T-lymphocytes and IL-6, show circadian variations, and corticosteroids may change this periodic oscillation. It has been demonstrated that giving glucocorticoids at night is more efficient in alleviating symptoms because it prevents the nocturnal surge of proinflammatory cytokines [88],[96]. This approach can regulate various inflammatory pathways, leading to better sleep and a decrease in depressive symptoms [88],[97]. Modified-release prednisone is a medication whose active ingredient is meant to be released gradually over time as opposed to immediately. Only a regulated delay in the release of the active component distinguishes the modified-release version from the original version [88],[89]. Taking the medication with meals decreases its bioavailability but does not affect its efficacy (Figure 4.9.) [98],[99].

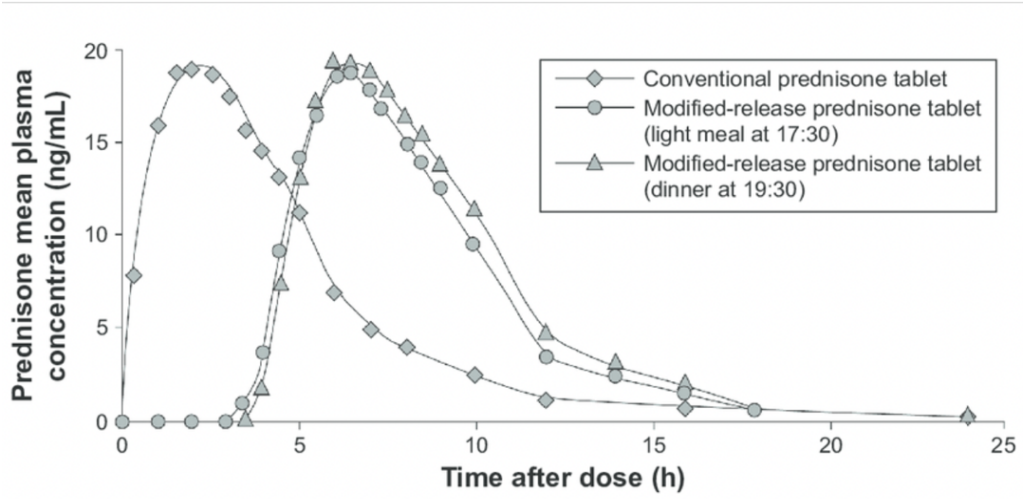

Figure 4.9. Prednisone pharmacokinetics of the regular and modified-release (MR) forms [98].

Modified-release prednisone is a new approach to low-dose prednisone chronotherapy in RA, delivering the medication at night (10 - 11 p.m.) and releasing prednisone between 2 and 3 a.m. (Figure 4.10.) [100],[101]. Compared to low-dose prednisone, which is usually administered between 7 – 9 a.m., this method is more successful in easing morning joint stiffness, RA patients' fatigue and resolving a purported HPA regulatory deficit as well as improving patient satisfaction [89],[102],[103]. Additionally, modified-release prednisone lowers the levels of the important inflammatory marker, IL-6, and reduces the requirement for biological DMARD treatment [88],[48],[104]. Switching from the standard morning dosage of prednisone to the modified-release version has no impact on adrenal function and continuous use of the modified-release form does not worsen adrenal impairment [89],[105]. Modified-release prednisone's PK characteristics help it work better to treat RA and lessen the need for higher doses and other therapies.

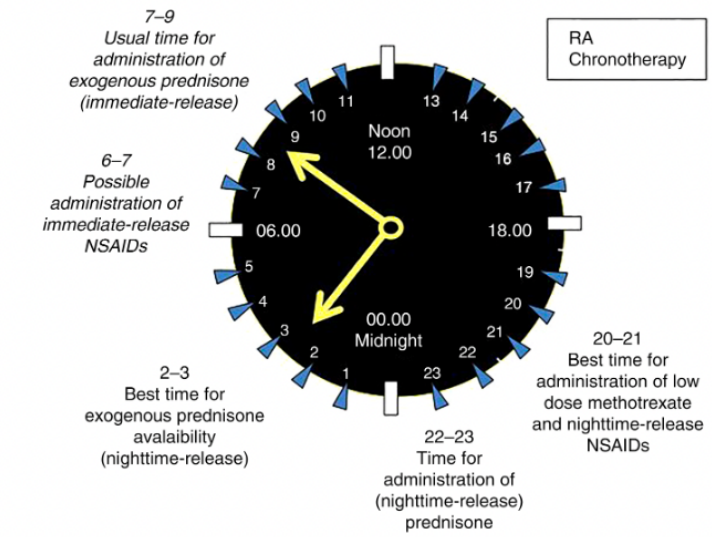

Figure 4.10. Chronotherapy in RA. Administration between 10 – 11 p.m. to release the active ingredient between 2 – 3 a.m. [88].

4.2.2.2. Conventional and biologic DMARDs

Methotrexate, leflunomide and cyclophosphamide are examples of conventional and biologic DMARDs that target the circadian activation of immune/inflammatory response cells. According to research, methotrexate is most effective when given based on the pro-inflammatory cytokine TNF-α's 24-hour cycle [88],[89]. When administered in time with TNF-α 's 24-hour cycle, methotrexate can reach its peak blood concentration during the hours when TNF-α levels are at their maximum, which are at night. This improves the ability to manage immune/inflammatory response cells and the effectiveness of treating the symptoms of RA. Furthermore, compared to conventional dosing methods, taking methotrexate at night has been shown to considerably reduce RA symptoms (Figure 4.10.) [88]. This is due to methotrexate's pharmacokinetics, which allows patients to achieve ideal plasma concentration levels of the medication by taking it before bedtime. Methotrexate is quickly absorbed in the digestive system and has a relatively long elimination half-life of 6 to 8 hours.

4.2.2.3. NSAID

Taking NSAIDs like indomethacin and flurbiprofen in the evening has been found to be more effective in treating morning symptoms of RA (Figure 4.10.). The reasons for this include optimal absorption and concentration of the drugs during the early morning hours, alignment with the body's circadian rhythm and peak inflammation period, potential improvement in sleep quality and individual variation in response to medications [89].

4.2.2.4. Drug delivery systems

Specialized delivery methods are required for scheduled drug administration at night, as traditional methods like oral pills or injections are unable to release drugs in a regulated manner. These specialized delivery forms have been developed to maximize the effectiveness of therapy for treating RA symptoms by timing the drug delivery to coincide with the circadian rhythm. They aim to enhance treatment efficacy while reducing adverse effects. Examples of these specialized delivery forms include oral compression-coated tablets, dual pulse multiparticulate dosage forms, mini-tablets-filled pulsincap, pH-triggered delayed-release colon-specific aceclofenac microspheres and eudragit-coated aceclofenac-loaded pectin microspheres [88],[89].

4.3. PHARMACOKINETIC MODELLING

The study conducted a comprehensive assessment of the effects of prednisone and prednisolone through both oral and IV bolus administration. For the oral administration simulation, a range of prednisone doses from 0 to 60 mg with 20 mg intervals was used. The simulation spanned a 24-hour clock time period, allowing for hourly intervals to compare different dosing times. This time frame was chosen to align with our natural circadian rhythm and reflect the conventional once-daily dosing regimen. The evaluation of prednisone's effectiveness and pharmacokinetics at steady state was based on parameters such as CCS%, AUEC% and C_{trough}. These measures provide insights into the extent of cortisol suppression achieved over time, the overall impact on cortisol levels and the lowest plasma concentration of the drug between doses. Similarly, the simulation for bolus IV administration involved prednisolone doses ranging from 0 to 100 mg with 20 mg

intervals. The time period and intervals used in this simulation were consistent with the oral administration simulation. CCS%, AUEC%, and C_0 were again utilized to evaluate the effectiveness and pharmacokinetics of prednisolone.

The study aimed to provide guidance for the optimal dosing strategies of prednisone and prednisolone in the context of conditions such as rheumatoid arthritis, where excessive inflammation and an overactive immune response are primary concerns. The objective was to achieve effective cortisol suppression and modulation of the immune response. Ideally, a high CCS% and AUEC% would be favourable as they suggest a substantial and consistent decrease in cortisol levels. These indicators signify effective management of inflammation and symptoms, highlighting successful control of the body's stress response. However, it is crucial to consider the potential risks associated with chronic exposure to prednisolone, as an abnormally high AUEC% may increase the probability of adverse events. Therefore, finding the right balance between drug efficacy and limiting overall exposure is essential. Monitoring C_{trough} is necessary to maintain adequate drug levels throughout the dosing interval, ensuring sustained therapeutic effects and consistent immunosuppression.

By analysing the contour plots (available in Appendix I), the findings revealed important patterns. Starting from an oral dosage of 30 mg of prednisone, the proportion of CCS% in the early morning hours (2 - 4 a.m.) approached 80-100%. Similarly, in the evening hours (9 - 12 p.m.), a slightly higher oral dosage of 40-50 mg achieved a comparable pattern of cortisol suppression. In contrast, significantly higher dosages were required to achieve a high percentage of CCS% when administered in the morning between 8 and 9 a.m., which is the current common practice. As a result, the CCS% remained modest (0-20%) at the usual dosages administered in the morning. However, no observable difference was noted in AUEC% between an evening or early morning dosage. Additionally, C_{trough} increased proportionally with the dosage, indicative of the known linear PK of prednisone. The outcomes for prednisolone administered via the IV route exhibited similar patterns.

5. DISCUSSION

The circadian rhythm, as previously mentioned, is the 24-hour internal biological cycle that regulates the sleep-wake cycle and other physiological processes in living organisms. It is controlled by the SCN, a group of specialised brain cells located in the hypothalamus. Circadian rhythm is influenced by both internal and external factors, such as hormones and metabolism, as well as external factors like temperature and light. Jetlag, shift work and exposure to artificial light at night can all disrupt the circadian cycle. Depression, trouble with sleeping and an increased risk of chronic diseases like diabetes, obesity and heart disease are all serious health effects of circadian rhythm abnormalities. The potential of chronotherapy to enhance treatment and boost patient outcomes has attracted more attention in recent years. Chronotherapy means matching therapies with a patient's circadian rhythm, which is the internal biological clock that controls hormone synthesis, the sleep-wake cycle and other physiological and behavioural processes.

Chronotherapy has emerged as a promising approach for the management of hypertension and rheumatoid arthritis by considering the circadian rhythm and timing of medication administration. In the case of hypertension, the evening/before bed treatment strategy in chronotherapy offers several advantages over morning treatment. Administering antihypertensive drugs in the evening can lead to improved BP management, reducing the risk of CVD and preventing organ damage to vital organs such as the kidneys, brain, eyes and heart (Figure 5.1.) [62],[54]. The individualization of medication based on the patient's circadian cycle is a key benefit of the evening treatment strategy, as it optimizes the effectiveness of the treatment by aligning medication administration with the patient's circadian rhythm. Furthermore, chronotherapy in the evening has shown to improve renal function, which is particularly significant considering the detrimental effects hypertension can have on the kidneys. By effectively managing blood pressure through evening treatment, the risk of kidney damage can be reduced. Additionally, studies have indicated that evening chronotherapy can result in decreased levels of LDL cholesterol and increased levels of HDL cholesterol, promoting better cardiovascular health and reducing

the risk of heart disease. Moreover, implementing the evening treatment strategy has been associated with a reduced risk of developing diabetes and CKD both of which are conditions closely linked to hypertension [57].

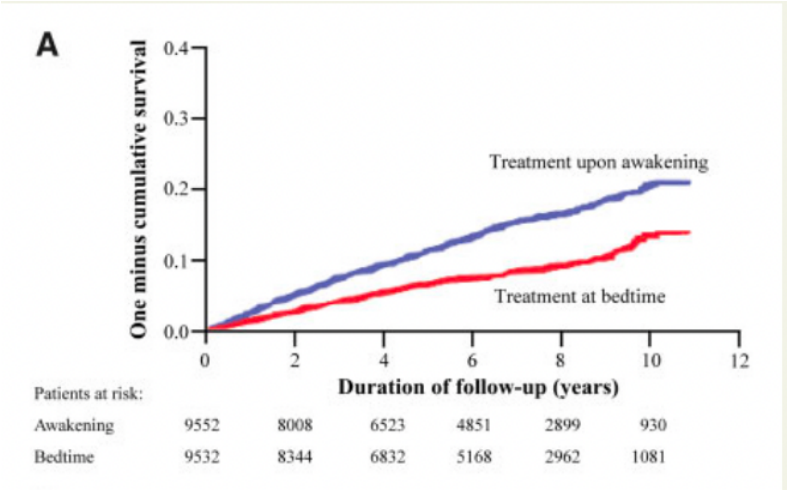

Figure 5.1. The Kaplan-Meier cumulative curve for CVD outcome based on the hypertension treatment schedule is shown. CVD outcomes include heart failure, peripheral artery disease, myocardial infarction, stroke, angina pectoris and CVD mortality [62].

However, implementing the evening treatment strategy in chronotherapy faces challenges due to the short duration of action of antihypertensive medications. Suboptimal drug concentrations the following morning can compromise BP management by falling below the MEC. To address this issue, various technologies, such as extended-release formulations, delayed-release formulations and chronotherapeutic drug delivery systems, have already been developed. These innovations aim to maintain therapeutic drug concentrations throughout the night and into the morning, ensuring optimal BP control and overcoming the limitations of short-acting medications. Additionally, some antihypertensive drugs can cause side effects that may disrupt sleep, such as frequent urination, nocturnal cough, or gastrointestinal issues. Taking these medications right before bedtime can increase the likelihood of experiencing these side effects during sleep, thereby potentially reducing the quality of sleep. Therefore, it is crucial to consider these factors when considering the application of chronotherapy.

Moving on to RA, chronotherapy has emerged as a promising approach for improving symptom management by targeting the nocturnal surge of proinflammatory cytokines. This targeted timing helps regulate inflammatory pathways, resulting in various benefits such as improved sleep quality, reduced depressive symptoms, alleviated fatigue and potentially resolving a purported HPA axis regulatory deficit. However, like in hypertension, traditional drug delivery methods are insufficient for administering medications at specific times during the nocturnal period, necessitating the development of specialized delivery systems. Examples include the oral compression-coated tablet, which ensures controlled and sustained release throughout the night, and the dual pulse multiparticulate dosage form, which offers sequential release to address the nocturnal surge of proinflammatory cytokines. The mini-tablets-filled pulsincap provides precise dosing by combining mini-tablets with a pulsincap capsule and pH-triggered delayed-release colon-specific microspheres targeted medication delivery to the colon during the nocturnal period. Finally, eudragit-coated aceclofenac-loaded pectin microspheres offer controlled release during the night, enhancing the efficacy of chronotherapy [88],[89]. Chronotherapy with prednisone/prednisolone administration in the evening or early morning has been found to induce the greatest cortisol suppression, aligning with the circadian rhythms of RA symptoms. This knowledge is essential in the treatment of diseases like RA, which often exhibit circadian patterns. Clinical investigations involving RA patients have demonstrated that administering prednisone/prednisolone in the early morning or late evening is associated with improved symptom management and decreased inflammatory activity [48].

Just like with antihypertensive drugs, some anti-rheumatic drugs can have side effects that disrupt sleep, such as gastrointestinal issues, nausea, vomiting, headaches, or dizziness. Taking these drugs in the evening can increase the chances of experiencing these side effects during sleep, thereby affecting the quality of sleep. Therefore, it is again crucial to consider these factors when considering the application of chronotherapy. Furthermore, certain anti-rheumatic medications, like NSAIDs, may lead to gastrointestinal problems, including stomach ulcers, pain, indigestion, or gastrointestinal bleeding. Taking these medications in the evening can heighten the risk of encountering such gastrointestinal side effects because they might linger in the digestive system throughout the night. In addition,

there are some anti-rheumatic medications that require ingestion with food to safeguard the stomach and enhance absorption. However, taking these medications in the evening can pose challenges in coordinating their intake with meals, particularly if one doesn't consume any food after dinner. This can make it harder to adhere to the necessary timing for medication and food consumption.

Optimizing medication administration for individuals with irregular sleeping patterns is a crucial challenge in chronotherapy. Shift workers, pilots and frequent travellers across different time zones could benefit from biometric-based systems that deliver medications based on demand. These systems can be activated by biomarkers such as circadian clock gene products, the RAAS, or other circadian biomarkers. By controlling nocturnal blood pressure surges and protecting vital organs, including blood vessels, kidneys and heart tissue, these systems can effectively manage hypertension and reduce the risk of CVD. To prioritize the development of real-time drug delivery systems for hypertension, it is important to respond to biomarkers indicating high BP and related health conditions. This approach aims to deliver a range of drugs targeting crucial BP control systems in an economical and efficient manner. By providing comprehensive therapy for irregular blood pressure patterns, these systems can enhance medication effectiveness, prevent end-organ damage and improve patient safety. Similarly, individuals with disrupted sleep patterns in the context of RA face challenges in adhering to the ideal medication timetable. To optimize the effectiveness of glucocorticoid chronotherapy for such individuals, modified approaches are required. Biometric-based systems that consider circadian biomarkers, such as the expression of clock genes or inflammatory markers, can be employed to deliver drugs at the appropriate time and mitigate nocturnal proinflammatory cytokine surges. Efforts should be directed towards developing innovative drug delivery systems for RA that respond to individualized circadian rhythms and deliver medications precisely when they are most needed. By incorporating real-time monitoring and biomarker-based activation, these systems can improve symptom management, alleviate fatigue, and enhance patient satisfaction. By addressing the challenges posed by irregular sleep-wake cycles in RA patients, these systems offer a promising solution for optimizing medication administration in this context [54].

Considering all the information presented, it can be concluded that further research is needed to establish the safety and efficacy of evening drug delivery in the management of hypertension and RA. While recent studies have demonstrated encouraging findings in terms of nocturnal administration's effectiveness in regulating blood pressure and managing symptoms, further extensive investigations are necessary to evaluate its long-term outcomes. Before implementing antihypertensive and glucocorticoid chronotherapy in the evening as a standard practice, randomized controlled trials (RCTs) with extended research durations are necessary. These trials should involve larger sample sizes and longer follow-up periods to evaluate the potential effects of nocturnal administration on important clinical endpoints such as cardiovascular events, mortality rates and quality of life outcomes. Furthermore, it is crucial to investigate the impact of various factors such as age, gender, race and comorbidities on patients' response to late night drug administration. Understanding how these variables influence the effectiveness and safety of evening chronotherapy will help tailor treatment approaches and optimize patient outcomes [41].

Due to the diversity of individual physiology and behaviour, chronotherapy application might be difficult to implement at an individual patient level. Since each person has a unique entrainment phase, some people are by nature more active and alert in the morning while others are in the evening. This resulted in the creation of chronotypes, which categorises people according to when they like to be awake and asleep. Understanding the differences in chronotypes, which vary from early morning to late at night, is crucial for optimising chronotherapy [8]. An individual's circadian rhythm can be influenced by things including genetics, age, weight, time zone, light exposure, sex and the environment. The dim light melatonin onset (DLMO) is one tool that may be used to determine an individual's internal clock time, although it can be expensive and requires many tests in a controlled environment. Consistently determining an individual's internal clock time at any given moment becomes challenging due to these factors [106]. Studies on chrono-effectiveness have been suggested as a solution to some of these problems. In such research, a different arm of clinical trials is used in which the medicine is administered at a time that is not consistent with the patient's circadian rhythm [3].

Another challenge is extrapolating results to human beings since most research has been done on nocturnal rats. The disparities between human and rodent circadian rhythm studies are progressively being addressed through in-silico research and the use of cutting-edge models, including primates and humanised animals [21]. The use of chronotherapy in clinical settings is not without its practical difficulties. For instance, comorbid patients may need different medication classes, making it difficult to adhere to dosage recommendations for chronotherapeutic formulations. Additionally, the time allotted for chronotherapy may be limited by pharmacy time restrictions. Numerous pharmacists acknowledge that they lack a thorough understanding of chronotherapy and emphasise the need for education and training to advance their knowledge. Chronotherapy has great potential advantages despite these difficulties. Chronotherapy can improve the efficacy of drugs and decrease potential negative effects by adjusting treatments to a patient's circadian cycle. However, further study is required to identify practical biomarkers that can track a person's circadian rhythm and to provide resources to assist pharmacists to apply this understanding to clinical practice in order to fully realise the potential of chronotherapy [8],[19],[106].

PK/PD modelling has shown promising results in a variety of therapeutic areas, still there is a lack of research on its application to RA and hypertension. Despite the widespread prevalence of RA and hypertension, little research has been done on the potential benefits of using PK/PD modelling to optimise drug dosage and improve treatment outcomes in these patient populations. Personalised therapeutic dosing based on PK/PD modelling may lead to optimised drug concentrations regarding chronotherapeutic effects and a lower risk of adverse effects. Further investigation is necessary to fully comprehend the potential of PK/PD modelling in the chronotherapeutic treatment of RA and hypertension. Future studies should focus on identifying the factors that influence individual drug responses and developing personalized dosing strategies. These endeavours hold the promise of enhancing therapeutic outcomes and reducing healthcare expenses through more effective and tailored therapies. Given the current research landscape and the potential advantages of chronotherapy, it emerges as a viable approach for managing not only RA and hypertension, but also other diseases. However, it is imperative to continue refining

chronotherapy protocols and exploring the application of PK/PD modelling to ensure safety, efficacy and optimal implementation for individual patients.

Based on the comprehensive review of the literature on the topic and the information presented in this master's thesis, it becomes evident that chronotherapy holds substantial promise and potential benefits for the management of RA and hypertension. The accumulated evidence suggests that aligning the timing of medication administration with an individual's circadian rhythm can significantly improve treatment outcomes, reduce health risks, and optimize therapeutic efficacy in these conditions. Several studies have demonstrated that the circadian rhythm plays a crucial role in the regulation of physiological processes, including the pathogenesis and progression of RA and hypertension. By considering the natural fluctuations in disease activity and symptom severity throughout the day, chronotherapy offers a promising approach to enhance the effectiveness of treatment interventions. Nevertheless, factors such as the availability of specialized drug delivery systems, side effects of evening dosing, feasibility of aligning medication schedules with individual circadian rhythms and the long-term safety and efficacy of chronotherapy need to be taken into consideration. RCTs with larger sample sizes and longer follow-up periods are necessary to evaluate the impact of chronotherapy on important clinical endpoints, such as cardiovascular events, mortality rates and quality of life outcomes. Additionally, the influence of various factors, including age, gender, race and comorbidities, on individual patient response to chronotherapy should be investigated to tailor treatment approaches and optimize patient outcomes.

In conclusion, based on the literature reviewed and the findings presented in this master's thesis, chronotherapy emerges as a relevant and potentially beneficial approach for the management of RA and hypertension. However, its implementation should be carefully considered on a case-by-case basis and supported by ongoing research and clinical evidence.

6. CONCLUSION

Chronotherapy has emerged as a crucial approach in enhancing treatment outcomes for hypertension and rheumatoid arthritis. Night-time administration of antihypertensive drugs has proven to be safe and effective, leading to improved 24-hour blood pressure profiles. In the management of rheumatoid arthritis, night-time drug administration demonstrates greater effectiveness compared to morning dosing. This can be attributed to the targeting of the nocturnal surge of pro-inflammatory cytokines. To fully realize the potential of chronotherapy, specialized drug delivery systems play a critical role. These systems enable precise and timed release of drugs, ensuring optimal drug administration aligned with the circadian rhythm. Controlled release systems, circadian-synchronized drug delivery and smart drug delivery systems all contribute to enhancing treatment outcomes. The ongoing development and utilization of specialized drug delivery systems will continue to advance the field of chronotherapy. While limited literature exists on PK/PD models for studying circadian rhythms and chronotherapy in hypertension and rheumatoid arthritis, incorporating PK/PD modelling holds promise for improving treatment outcomes. A PK/PD model employed in this thesis examined the effects of prednisone/prednisolone delivery timing and dosing intervals. The study findings suggest that the PK/PD of prednisone/prednisolone can be influenced by the timing of administration, particularly during the late night or early morning allowing for lower doses resulting in similar efficacy but potentially better tolerability.

In summary, chronotherapy in hypertension and rheumatoid arthritis holds significant potential for improving treatment outcomes by considering the circadian rhythm and timing of drug administration. The adoption of evening treatment strategies in hypertension offers various advantages. The challenges associated with short-acting drugs are being addressed through the development and implementation of specialized drug delivery systems. Similarly, in RA, chronotherapy effectively targets the nocturnal surge of pro-inflammatory cytokines, resulting in symptom alleviation. By further advancing our understanding of mechanistic PK/PD models and incorporating them into clinical research and practice, we can unlock additional potential for optimizing treatment outcomes.

7. BIBLIOGRAPHY

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8. APPENDICES

8.1. APPENDIX I

8.1.1. Excel spreadsheet

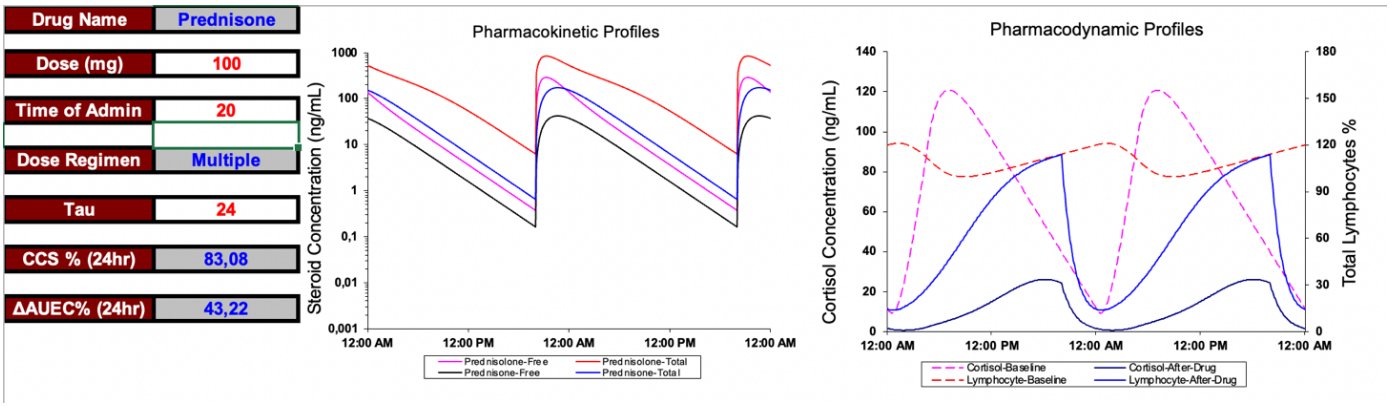

Figure 8.1 Example of the Excel spreadsheet for oral administration of prednisone [48].

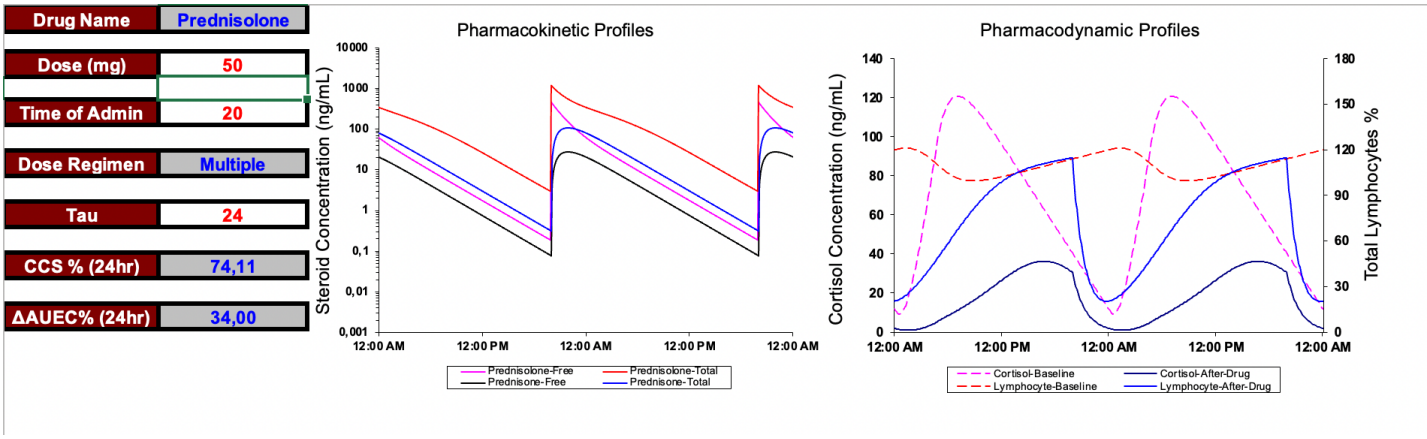

Figure 8.2. Example of the Excel spreadsheet for IV administration of prednisolone [48].

CCS% (24hr): cortisol suppression over 24 hours compared with baseline ΔAUEC% (24hr): alteration of total lymphocyte trafficking over 24 hours compared with baseline; Pharmacokinetic Profiles: free and total prednisone, free and total prednisolone concentration-time profiles; Pharmacodynamic Profiles: plasma cortisol concentration and alteration of plasma total lymphocyte trafficking (%) time profiles at baseline and after treatment with prednisone or prednisolone.

8.1.2. Contour plots regarding oral administration of prednisone

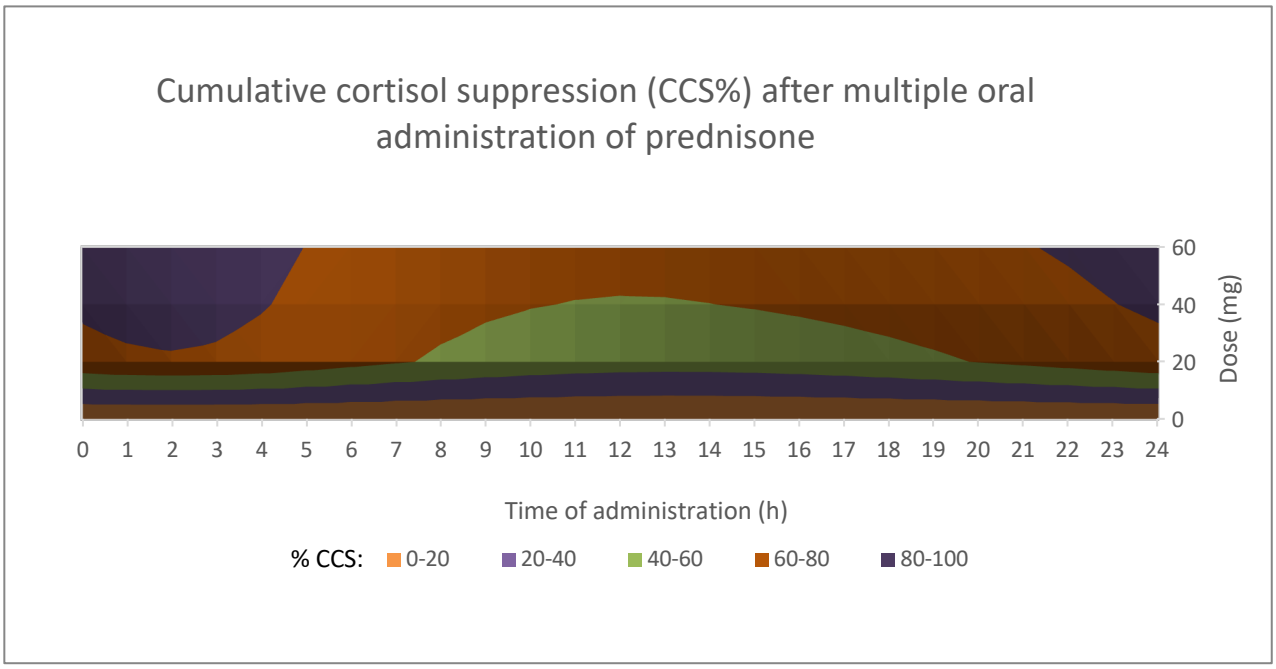

Figure 8.3. Cumulative cortisol suppression (CCS%) after multiple oral administration of prednisone. Legend: percentages CCS from 0 – 100%. Created in Excel.

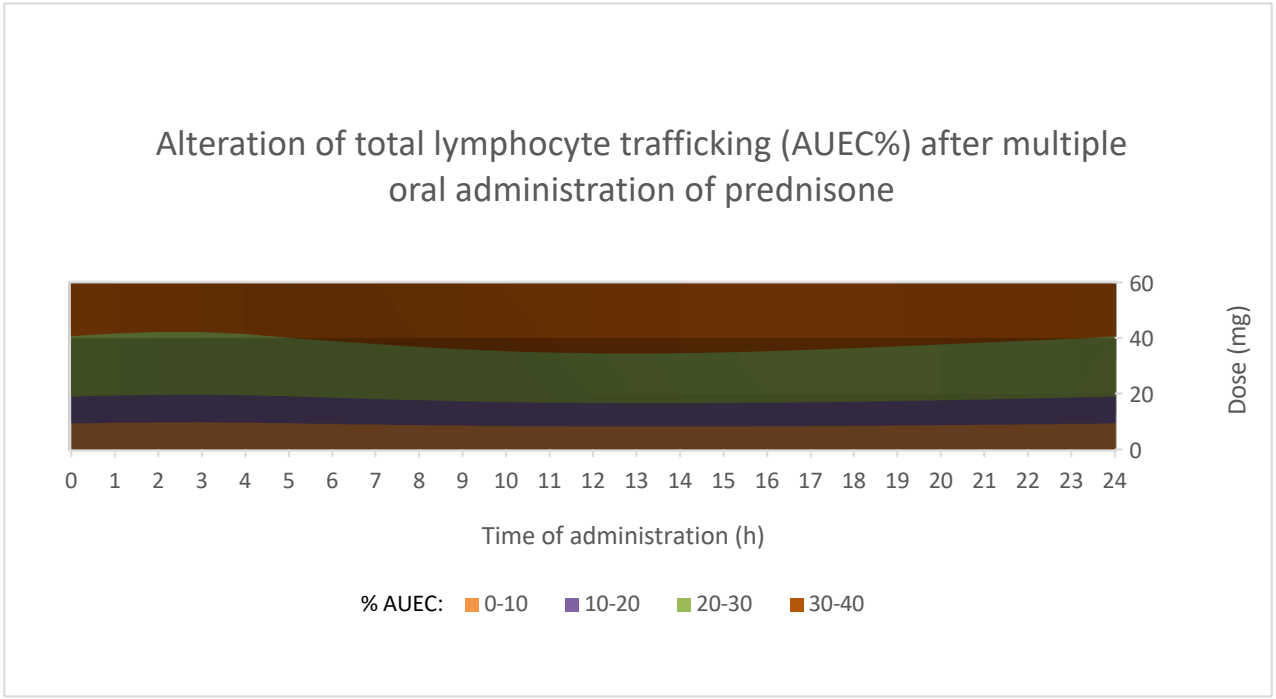

Figure 8.4. Alteration of total lymphocyte trafficking (AUEC%) after multiple oral administration of prednisone. Legend: percentages AUEC from 0 – 40%. Created in Excel.

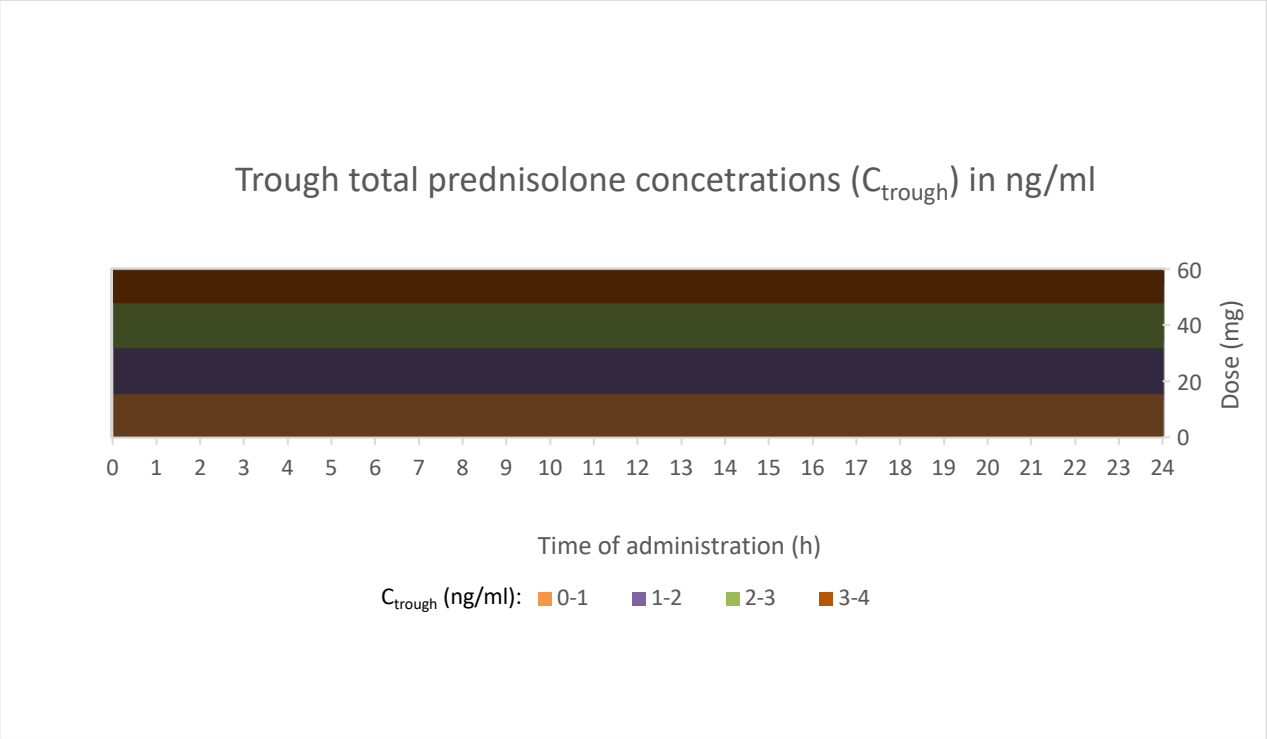

Figure 8.5. Trough total prednisolone concentration (C_{trough}). Legend: C_{trough} from 0 – 4 ng/ml. Created in Excel.

8.1.3. Contour plots regarding IV administration of prednisolone

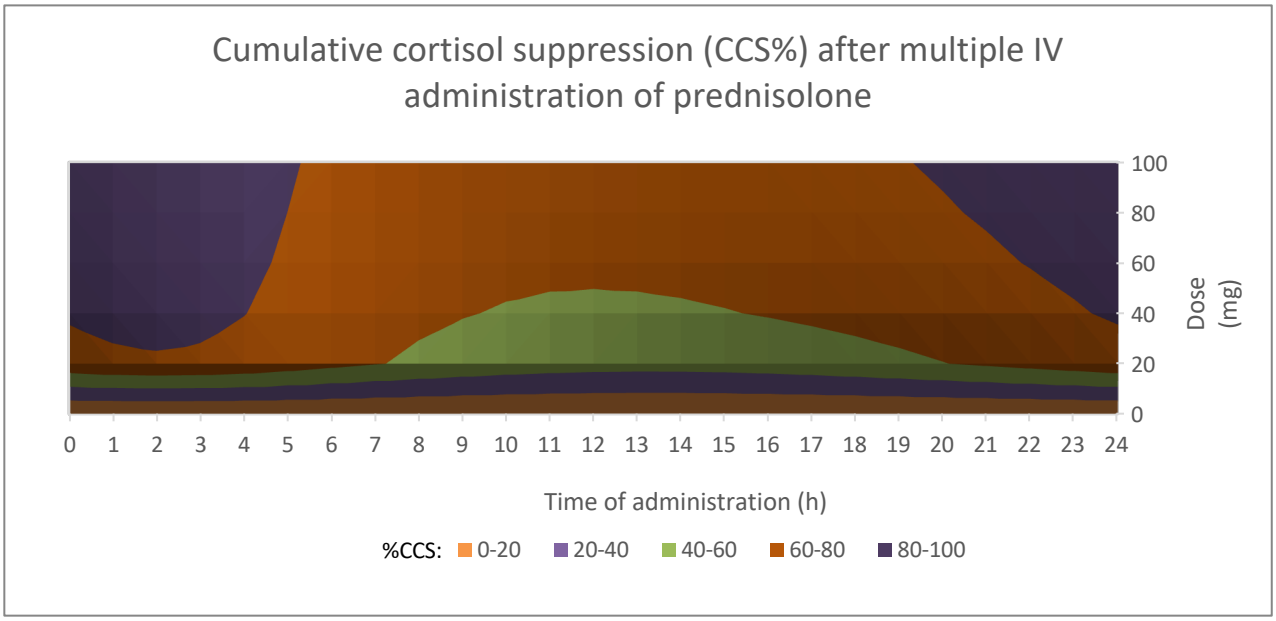

Figure 8.6. Cumulative cortisol suppression (CCS%) after multiple IV administration of prednisolone. Legend: percentages CCS from 0 – 100%. Created in Excel.

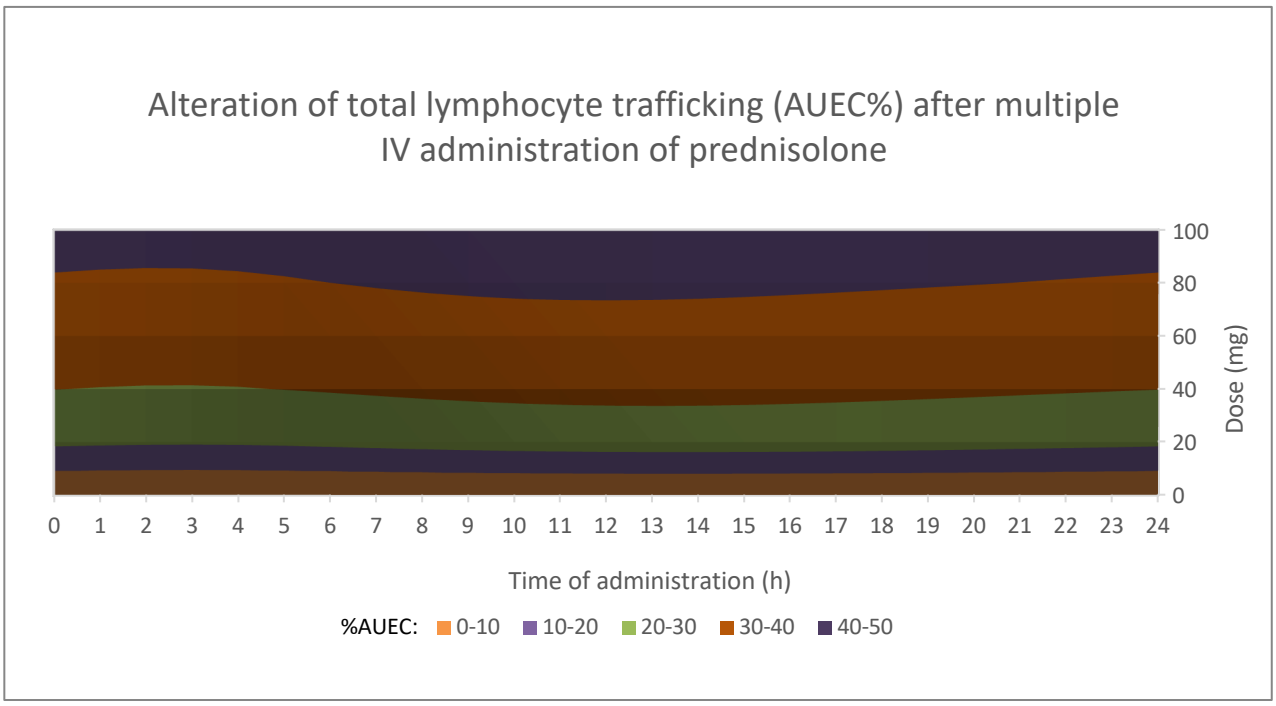

Figure 8.7. Alteration of total lymphocyte trafficking (AUEC%) after IV administration of prednisolone. Legend: percentages AUEC from 0 – 50%. Created in Excel.

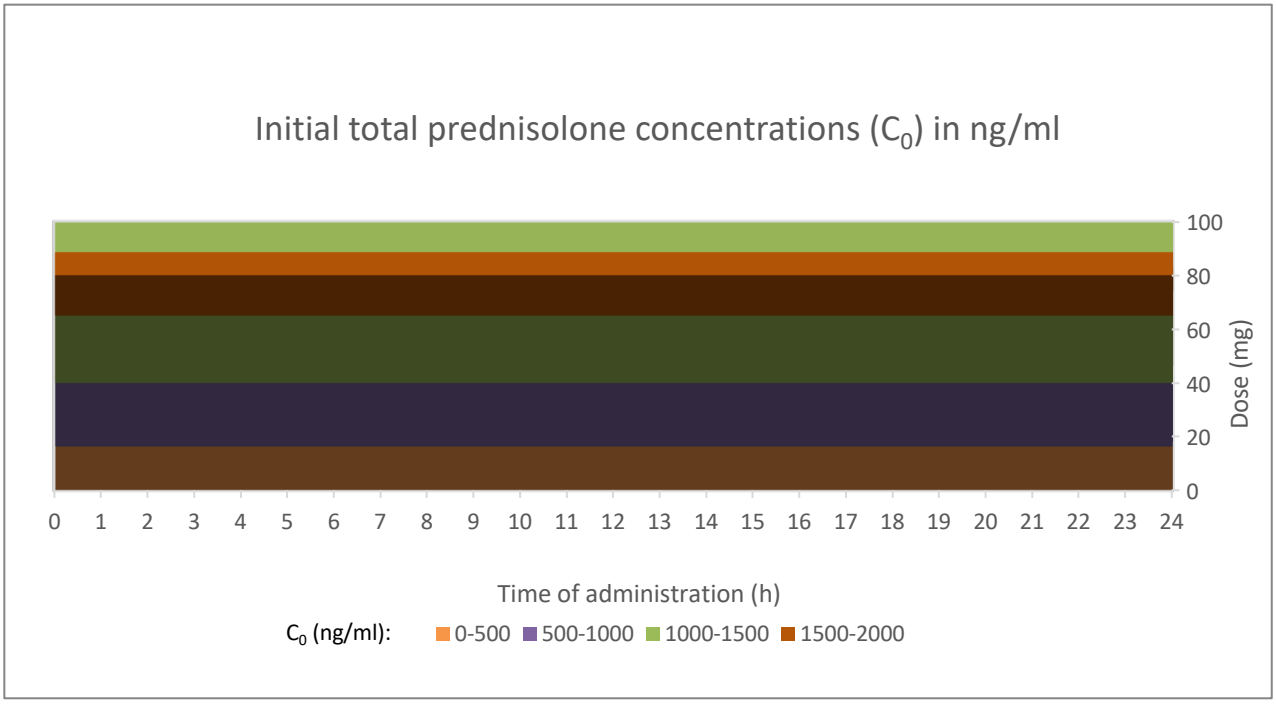

Figure 8.8. Initial total prednisolone concentrations (C_0). Legend: C_0 from 0 – 2000 ng/ml. Created in Excel.

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