ORIGINAL RESEARCH

In Vitro Modeling of Agranulocyte Migration in Response to Circadian Rhythm

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Abstract

Background: The circadian rhythm, an intrinsic biological clock, regulates various physiological processes, including immune function. Agranulocytes, such as lymphocytes and monocytes, are key components of adaptive immunity, but their behavior in response to circadian influences is not fully understood. This study examines the impact of circadian rhythms, specifically light-dark cycles, on the migration of agranulocytes in vitro.

Objective: To investigate the rate of agranulocyte diffusion in response to circadian light and dark cycles using an in vitro migration model.

Materials and Methods: An in vitro diffusion chamber was designed using a nitrocellulose membrane to simulate agranulocyte migration. Blood samples from healthy individuals were processed to isolate lymphocytes using density gradient centrifugation. The isolated lymphocytes were cultured in the migration chamber under alternating 12-hour light and dark cycles for three days. Cell counts were obtained using a Neubauer chamber at the start and end of each cycle.

Results: A higher migration rate of agranulocytes was observed during the dark cycle compared to the light cycle. Across three days, the average difference in cell counts between light and dark cycles was 2000–2500 cells per cubic millimeter. This indicates that lymphocyte migration is more pronounced during the dark phase of the circadian cycle.

Discussion: The results suggest a circadian rhythm dependency in agranulocyte migration, aligning with existing evidence that immune responses and cellular processes are influenced by internal biological clocks. Disruption of circadian rhythms has been associated with altered immune responses and increased susceptibility to diseases, underscoring the importance of these findings.

Conclusion: The circadian rhythm, particularly the light and dark cycles, significantly impacts agranulocyte migration rates. Further research is needed to explore systemic and molecular mechanisms driving these changes and their implications for immune function and disease management.

Keywords: Agranulocytes, lymphocytes, circadian rhythm, immune response, in vitro modeling

Introduction

Blood constitutes a major component of the human body. An adult human body comprises of 5 litres of blood. The main components of blood include plasma and blood cells which can be further subdivided into red blood cells, white blood cells and platelets[1]. White blood cells are divided into granulocytes and agranulocytes. The granulocytes are composed of eosinophils, neutrophils and basophils. Agranulocytes comprises of monocytes and lymphocytes which are devoid of cytoplasmic granules with a non segmented nucleus. Lymphocytes are further divided into B lymphocytes and T lymphocytes. The agranulocytes have a lymphoid origin and constitute 35% of total leukocytes. They play a major role in adaptive immunity ie, they are capable of eliciting an immune response to a particular pathogen[2].

Blood cells circulate within the reticulo-endothelial system; however, a significant proportion of white blood cells reside within tissues, where they carry out their primary immunological roles. They diffuse across the blood vessels in their own way. They soften their large nuclei, push them to the front of their cells, and then use their probes to break

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away the blood vessel wall, scaffolding to pass through[3,4].

Circadian rhythm is the internal clock controlled by our brain which regulates the sleep and wake cycle in response to day and night changes in the surrounding environment. This internal clock helps our body in adapting to the changes in the surrounding environment, temperature, radiation and food availability. Studying the connection between the human body's circadian rhythms and cellular biology is crucial to comprehending the pathophysiology and underlying physiology of various disorders. An organism's health and cellular function can be negatively impacted by changes in age, environment, or genetic makeup[5].

The light-dark cycle is an essential regulator of circadian rhythms, which influence various physiological processes in organisms based on environmental signals. Light acts as the primary "zeitgeber" (time cue), synchronizing the body's internal clock housed in the suprachiasmatic nucleus (SCN) of the brain. Specialized retinal cells respond to light stimuli, transmitting signals to the SCN to adjust or align the circadian rhythm. Morning light exposure tends to shift the circadian rhythm earlier, promoting alertness and wakefulness, while exposure to light in the evening—particularly from artificial sources—can delay the rhythm. This delay can inhibit melatonin secretion, a hormone necessary for signaling nighttime, thereby disrupting sleep quality. In contrast, darkness supports the body's natural restorative processes, enhancing sleep and immune function. Disruption of the light-dark cycle, such as through shift work or prolonged exposure to artificial light at night, can desynchronize circadian rhythms, leading to adverse effects on sleep, mood, and overall health. Proper regulation of light exposure is crucial for maintaining circadian alignment and overall well-being[6].

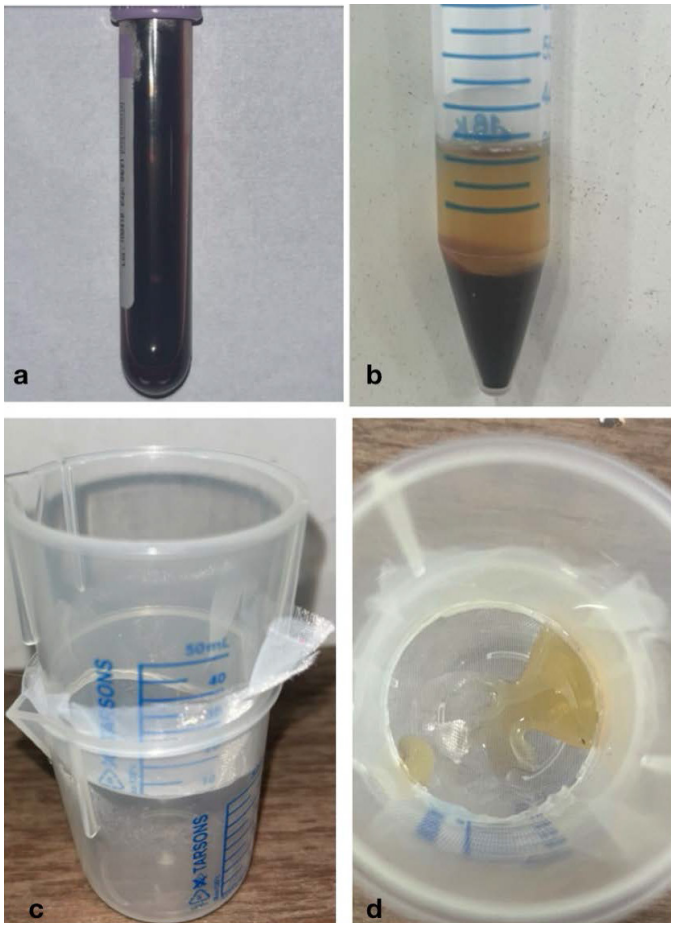

Fig. 1: A) Sample blood, B) post centrifuge, C) diffusion apparatus D) membrane filter

This study aims to study the rate of diffusion of lymphocytes in response to circadian rhythm that is the light and dark cycle.

Materials and Methods

An artificial lymphocyte migration chamber was created by placing a filter medium with a membrane of Nitrocellulose between the upper and lower compartments (Figure 1). Freshly extracted blood was collected and diluted using balanced salt solution. 4 ml of blood taken and 2 ml of ficoll was added to it and centrifuged for 10 mins. Three layers were formed. Upper layer - plasma, middle layer - buffy coat, lower layer - RBCs. The buffy coat was separated and infused with growth media and launched into the artificially created diffusion chamber. The number of cells were calculated using Neubauer chamber prior to addition of growth media.

The apparatus was left undisturbed for a 12 hour light and dark cycle each, rates of diffusion was observed and cells were counted using the Neubauer chamber at the end of every 12 hours (after completion of both light and dark cycle respectively).

Results

Time	Day 1 (No. of cells per cubic mm)	Day 2 (No. of cells per cubic mm)	Day 3 (No. of cells per cubic mm)
At the end of 12 hour light cycle	6000 cells/ cub mm	7500 cells / cub mm	4500 cells / cub mm
At the end of 12 hour dark cycle	8500 cells/ cub mm	9000 cells / cub mm	6700 cells / cub mm

The number of cells per cubic mm were counted using a Neubauer chamber and the results were tabulated. From the results of the study it can be inferred that the number of cells diffused was greater at the end of the 12 hour dark cycle. On all three days observed the number of cells was more at the end of the dark cycle when compared to the 12 hour light cycle. The difference in number of cells ranged around 2000 - 2500 cells per cubic mm, when the blood samples were taken from normal healthy individuals.

Discussion

The phenomenon of migration of blood cells is important in several aspects for normal homeostasis. A study done on dendritic cells of skin revealed the mechanisms between cutaneous dendritic cells draining into the skin and lymphatic vessels. This plays a vital role in initiating adaptive immune responses[7]. Normally a process called transendothelial migra-

tion takes place in the endothelial lining wherein the cells from the circulation migrate to the site of inflammation in response to stimulus[8].

Nitrocellulose membranes have stable physical and chemical properties, along with high hydrophilicity and flexibility. These porous membranes have been applied as electrophoresis films, osmosis, ultrafiltration, and microporous membranes in the biological domains[9]. Additionally, the porosity of these membranes is crucial for cell adhesion and growth. Nitrocellulose membranes have a special three-dimensional microstructure that resembles a sponge and is advantageous for cell absorption, metabolism, and nutrient transfusion[10, 11].

Circulating lymphocyte counts oscillate on a daily basis in healthy humans, and all lymphoid organs and immune cell types tested to date harbor functional clocks[12]. The disruption of clock function can cause a shift in macrophages to secrete high amounts of cytokines, leading to inflammation. Furthermore, the circadian clock regulates cytokine secretion, which is important for immune responses, and plays a critical role in immune functioning[13]. Disruption of either the circadian rhythm or sleep can lead to inflammation and compromise the immune system, making organisms more susceptible to disease. Therefore, understanding the relationship between circadian rhythms, sleep, and immune function has important implications for public health and medicine[11].

The number of lymphocytes in lymph nodes and lymph fluctuates throughout the day. Rhythmic expression of Ccr7 and S1pr1 drives rhythmic lymphocyte homing and egress. Adaptive immune responses to infections and vaccinations are time-of-day dependent. Rhythmic adaptive immune responses are ablated when lymphocytes lose their circadian clocks. Most notably, lymphocytes invaded Lymph Nodes during the beginning of the night phase and left the tissue throughout the day. Weeks after vaccination, this led to oscillatory cell counts in lymph nodes, lymph, and variations in the adaptive immune response based on the time of day[14].

Circadian rhythms are biological processes synchronized with the Earth's light-dark cycle, regulated by the suprachiasmatic nucleus (SCN) in the brain. Controlled by clock genes such as PER and CLOCK, these rhythms govern sleep-wake cycles, metabolism, and temperature, with disruptions linked to conditions like metabolic disorders, aging, and cancer. Recent research highlights their role in maintaining physiological balance and their involvement in non-circadian functions, emphasizing their significance in health and disease[15].

Studies conducted on demonstrating circadian gating of immune responses weeks after the initial stimulus, maintained the time-of-day dependent differences. The number of cells in the lymph node and the early hours of the adaptive immune response appear to be crucial in controlling the strength of the reaction, which is not something that can be made up for by the following circadian cycle[16]. The body uses lymph nodes and lymph to transport lymphocytes, which is a crucial immune surveillance system. Research shows that adaptive immune responses are also time-of-day dependent and are eliminated when T cell circadian clock function is lost and that this trafficking happens in a circadian fashion[16]. The expression of factors specific to a given lineage and tissue determines the time of day at which major leukocyte subsets traffic in a rhythmic manner. This impacts the leukemic tumor burden and the inflammatory response, which in turn affects how human primary lymphocytes migrate[17].

A study conducted on oral squamous cell carcinoma revealed increased expression of CD8+ T cells and CD57+ NK cells in oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC), with the highest levels in OSCC. Thereby stressing the importance of immune cells in cancer[18]. A systematic review conducted on role of haematological parameters in oral cancer revealed that hematological parameters like neutrophil-lymphocyte ratio, lymphocyte-monocyte ratio, and platelet-lymphocyte ratio can predict long-term prognosis in OSCC patients[19].

Study conducted on assessing the work ability of construction workers using the Work Ability Index (WAI) and its correlation with oral hygiene, sleep quality, and shift schedules. The results revealed that a shift based working system is detrimental to health resulting in poor sleep quality and that measure should be advocated to ensure proper sleep physiology in order to maintain circadian rhythm, which can improve work efficiency[20]. A study conducted among college students assessing the level of awareness on sleep deprivation revealed most participants were aware of sleep deprivation, but many still suffered from it, highlighting the need for educational seminars to stress the importance of proper sleep cycle and maintaining circadian rhythm[21].

Conclusion

Thus the light and dark cycle play a vital role in affecting the rate of diffusion of leukocytes. Further studies can be done on systemic factors which affect the rate of diffusion of white blood cells along with circadian rhythm.

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