The meaning of fever rhythmicity and its relation to the timing of Plasmodium reproductive cycles during malaria infections.

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Introduction

Malaria is an infectious disease caused by parasites of the genus Plasmodium, mainly found in tropical regions. The parasites are transferred via mosquito bites and infect vertebrates, including humans. Symptoms arise 8 - 30 days after infection and are very similar to those of common infections, including headaches, fever and nausea. Clinical deterioration usually appears 3–7 days after onset of the fever. Complications include coma (cerebral malaria), organ failure, pulmonary oedema, metabolic acidosis and hypoglycemia (Trampuz et al., 2003). Although most of the (early) symptoms are not unique to malaria infections, its rhythmic fever is a well-known symptom. The period of fever cycles varies between species, but it's always a multiple of 24 hours. It has been well documented that the fever cycle is closely linked to the developmental cycle of the infecting parasite. Current treatment consists of combinations of artiseminin based medicine and often fever treatment (paracetamol or ibuprofen). Several studies after the 90s show that the effectivity of different treatments differs per developmental stage, suggesting that chronotherapy (timed treatment of medicine based on the peak of affectivity) could be a viable option.

To see what the possibilities for chronotherapy treatment are, I will try to answer the following questions in this thesis. Firstly: What causes the rhythmicity of both the developmental cycles and related fever? Secondly: what are the benefits of rhythmic fever (or rhythmic development) for the parasite and the host? And finally: what are its implications for treatment?

I will first explain a couple of things about temperature regulation in general and more specifically febrile temperatures. Then I will describe malaria and the organisms that cause it. Finally I will discuss the questions posed earlier.

Body temperature regulation

The physiology of thermoregulation has been studied thoroughly. The mechanisms regulating the temperature of one or several body regions keep the body temperature within a restricted range (Cabanac, 1975). In humans the range is generally between 34.4 and 37.8 C (Sund-Levander et al., 2002), depending on the region being measured. Surface temperature for example is generally lower than core body temperature.

Information about body temperature is acquired by temperature sensors (found on the body surface and in the core). They carry information on temperature to the hypothalamus and the thalamus. Several studies have tested and shown the abilities of the hypothalamic sensors to instigate a full range and pattern of thermoregulatory responses (autonomic and behavioral). And that the intensity is in proportion to the intensity of the stimulus (Williams et al., 1971 and

Refinetti et al., 1986). The preoptic area of the hypothalamus (POAH) appears to be most sensitive to these stimuli, although the whole base of the brain seems to be capable of reacting with instigating thermal stress responses.

Vasomotor responses are the cheapest of all thermoregulatory reactions, since the only energy expenditure is in the form of cardiac work (only a small portion of the total energy expenditure)**.** Studies wherein subjects were submitted to a heat load and simultaneously to a negative pressure of the lower body show that the need for temperature regulation appears to have priority over circulatory equilibrium (Heistad et al., 1973). Water excreted by salivary or sweat glands can also be used as an evaporative coolant. Besides these physiological responses, behavior is an important way to regulate temperature. These behaviors can range from simple (moving to a cooler/warmer environment) to more sophisticated (washing etc.) (Carlisle et al., 1973).

Local stimulation of the POAH has been shown to result in behavioral and physiological responses (Refinetti et al., 1986). Lesions in the same area cause severe impairment of the thermoregulatory responses to changes in environment temperature.

The existence of a circadian (approximately 24 hours) rhythm of core body temperature has been known for decades. In humans, temperature generally rises from a nighttime low point of approximately 36.5 C and reaches its peak of 37.4 C in the early evening (depending on the subjects rhythm), then falls to 36.4 C in the early morning (a couple of hours before waking) (Refinetti & Menaker, 1992). So far, most studies suggest that this circadian rhythm of body temperature results from an oscillation of the thermoregulatory setpoint (Aschoff, 1970). The suprachiasmatic nuclei of the hypothalamus (SCN) is thought to be the anatomical locus of the circadian pacemaker. (Refinetti & Menaker, 1992).

Fever regulation

A very specific part of thermoregulation is the regulation of fever. Fever (pyrexia) is characterized by an elevation of body temperature above the normal range of 36.5 – 37.5 ®C most likely due to an increase in the body's regulatory set-point which can be evoked by changes in the neural activity of the POAH. (Conti et al., 2004). Fever is a well-known symptom of many clinical disorders, particularly resulting from infection, and is often used as a diagnostic feature. It can be instigated via several pathways, however most involve specific cytokines. Endogenous cytokines are essential components of the immune system, produced by immuno-competent cells. Their primary function is the regulation and coordination of immune responses, locally, systematically and in the CNS. Certain cytokines (e.g. IL-1, IL-6 IL-1ra, IL-10, and TNF-a) can act as pyrogenic and antipyretic agents and play a pivotal role in inducing and

regulating fever-responses (Rothwell et al., 1995).They are thought to act on the POAH either directly, by affecting afferent neurons (Romanovski et al., 2000) or by enhancing the secretion of other proinflammatory chemicals. Cytokines like IL-1 and TNF are known to be produced in the periphery as well as in the brain and there is evidence to suggest the existence of carrier-mediated transport of specific cytokines in to the brain (Conti et al., 2004). Other studies suggest that the afferent fibers of the vagus nerve can be activated by cytokines and affect the POAH (Blatteis et al., 1997).

In short: cytokines are believed to directly and indirectly affect neuronal function of the POAH, and to participate in local inflammatory processes. This is thought to result in a change in thermoregulatory setpoint. The POAH then regenerates a systemic response resulting in a rise in body temperature to match the new set-point. How exactly this change in setpoint is achieved has yet to be discovered. However, the interaction between the immune system and the SCN is undoubtedly an important feature of the host response to injury or infection (Conti et al., 2004).

Function of fever

The exact functions of fever are mostly unknown. Small elevations in body temperature (for example during fever) can potentially enhance the immune system. Increased metabolic rates as a result of fever can increase the mobility and activity of white blood cells, stimulation of cytokine production and activation of T Lymphocytes. Several studies show that elevated temperatures found during fever bouts can speed up neutrophil migration and enhance the secretion of cytokines and other chemicals related to the immune system (Bernheim et al., 1978). Other studies show a correlation between fever and decreased mortality rate (in various infections) (Weinstein et al., 1978, Lell et al., 2001). Although not all fever is beneficial (extreme fevers can be dangerous) in most cases it is likely that fevers serve to enhance the host defenses (Kluger, 1985)

Circadian rhythm of fever

Very little is known about the periodicity of fever. However, biological rhythms in multiple frequencies are found throughout the immune system (Haus et al., 1999). The inflammatory response is regulated in its intensity through the neuroendocrine and the immune systems and characteristically follows rhythmic patterns. The production of cytokines (with pyretic or antipyretic properties) IFN-y, TNF-a, IL-1 and IL-12 in humans appears to have a circadian rhythm, its peak generally occurs during the night or early morning. Since many of these

cytokines are also involved in fever regulation it is conceivable that fever intensity itself might show signs of rhythmicity. However, no data can be found on the rhythmicity of fever not induced by specific disorders or infections.

(Cabanac 1974) shows that circadian variations in body temperature remain intact during bouts of fever related to malaria. This suggests that fever itself is a change in setpoint rather than a change in thermoregulatory processes. It is possible that in general, the occurrence of fever is not really rhythmic, but that the intensity is, as a result of variability in cytokine presence throughout the day.

In short Fever is part of the immune response, and is most likely an elevation of the body temperature setpoint. Several cytokines are known to be able to regulate the fever response, by working directly on the hypothalamus. Fever thought to enhance the immune response and results in a higher activity of both immune cells and cytokines. Although it is currently unknown whether fever has an inherent rhythmicity, various cytokines have a circadian rhythmicity in production peaks.

Malaria

There are a number of diseases that can result in rhythmic fever. Probably the best example of this is malaria, in which fever cycles with periods of multiples of 24 hours can be found in the vast majority of cases (Hawking, 1970).
Malaria is an infectious disease caused by parasites of the genus Plasmodium. Humans can be infected by five different species: Plasmodium *vivax*, *knowlesi* (originally only infected monkeys, but is increasingly infecting humans in forested regions of South-East Asia), *ovale*, *malariae* and finally *falciparum* (most common in cases of malaria). The parasites and their vector (a specific species of mosquito) can be found in tropical regions (e.g. Africa, India, and South America). Although it is difficult to estimate the number of infections each year, due to poor health care in remote areas, according to the WHO an estimated 655.000 patients died in 2011 worldwide.

Early symptoms of plasmodium infection are nonspecific and very similar to regular systemic viral infections. They include headaches, fatigue, muscle- and joint aches, followed by fever, chills, perspiration and vomiting. At this early stage, patients can make a fast and full recovery when provided with prompt and effective treatment. When the infection remains untreated (due to ineffective medicine or delayed treatment) the disease can develop into severe malaria often manifesting coma (in cases of cerebral malaria), metabolic acidosis, severe anaemia hypoglycemia, acute renal failure or acute pulmonary oedema. During this stage, case fatality in patients in treatment is generally 10-20%. If left untreated, severe malaria is fatal in the majority of cases. (WHO, guidelines for the treatment of malaria, 2010)

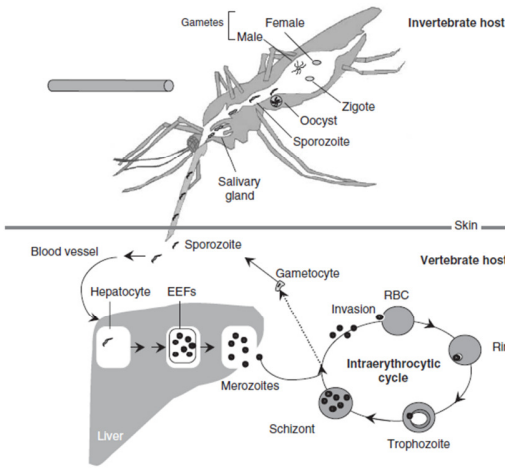

Figure 1: schematic presentation of the sexual and a sexual reproductive cycles of Plasmodium parasites. Garcia et al., 2001

The several stages of the disease can be connected to the events in development of the parasite. Infection is initiated by a bite of the female Anopheles mosquito, injecting saliva containing sporozoites (mature parasites, also called schizonts). Once in the bloodstream the sporozoites invade hepatocytes, reproduce asexually and release approximately 30.000 merozoites within 10-12 days. While the parasites reside in the liver, symptoms are nonspecific as mentioned above. It is only after the merozoites have been released that the pathogenicity becomes apparent. The mass release of merozoites into the bloodstream is called schizogony. After release, the merozoites invade erythrocytes (red blood cells) where they mature through different stages, termed ring-stage, trophozoite and finally schizont. Once the parasites reach the schizont (mature) stage, the asexual cycle starts over again (figure 2). The length of this cycle varies per species, Plasmodium *falciparum*, *ovale* and *vivax* shows a duration of approximately 48 hours, *malariae* shows 72 hours. At this stage symptoms like rhythmic fever etc. arise (Garcia et al., 2001).

A number of merozoites do not invade erythrocytes, but differentiate into male or female gametocytes, which can be taken up by feeding mosquitos. Within the mosquito, the gametocytes reproduce sexually and give rise to sporozoites, which in turn travel to the salivary glands in where they can easily be injected into the next host with the next blood meal (Enserink, 2000).

If left untreated the infection will rage on and enter a stadium called severe malaria which is often fatal (WHO, guidelines for the treatment of malaria, 2010). Plasmodium *falciparum* entices red blood cells to express adherents (surface proteins used to adhere to the artery/vein walls) which can result in blockages and eventually brain damage or organ failure (Udomsangpetch et al., 2002). The depletion of red blood cells caused by the parasites (hemolysis) causes severe problems for oxygen supply throughout the body and puts an extra strain on the lungs.

The diagnosis of malaria is based on the detection of parasites in the blood and the symptoms present. As mentioned earlier, the symptoms are non-specific, therefore diagnosis based on clinical features alone is very inaccurate and often results in over-treatment. A combination of fever (or a history of fever) and the presence of parasites in the blood stream has proven to be an effective method of diagnosis. High sensitivity of diagnosis in endemic areas is particularly important for the most vulnerable population groups, such as young children and non-immune

population. Meanwhile a highly accurate diagnosis can reduce the number of false positives, and thereby reduce unnecessary treatment with antimalarials.

Most of the countries affected have progressively updated treatment from chloroquine and sulfadoxine-pyrimethamine, which have become ineffective. Currently the recommended treatment is artemisinbased combination therapies (ACTs). Unfortunately due to the relatively high costs, implementation of these policies has lagged behind. With effective malaria control (population-wide vector control and large-scale deployment of ACTs), the number of malaria infections could be greatly reduced.

Resistance to antimalarial medicines has been documented in all classes of antimalarials, including artemisinin derivatives, and is a major threat to malaria control. The large-scale and indiscriminate use of antimalarials results in a strong selective pressure on malaria parasites to develop resistance. By combining different antimalarial medicine which work through different mechanisms, resistance onset can be slowed considerably, whilst still ensuring high cure rates (WHO, guidelines for the treatment of malaria, 2010).

Rhythmic fever and synchronized developmental cycles

Rhythmic fever is a very consistent symptom of Plasmodium infections and can be used in diagnosis. Periods differ per species, but it is always a multiple of 24 hours. So what causes this rhythmicity?

Fever resulting from Plasmodium infections generally occurs through a plateau of elevated temperature, interrupted by a (slight) decline right before a peak (fig. 2). The fever often starts initially quotidian or irregular, after a while it becomes a more regular rhythm. (Landau et al., 1991)

Hawking (1968) was one of the first scientists to describe and research the resemblance of the malaria fever cycle to a circadian rhythm and to link the fever pattern to the Plasmodium life cycle. He also pointed out that the rhythmicity was not a unique feature of human infecting parasites, but that other plasmodium species show similar life-cycle rhythms.

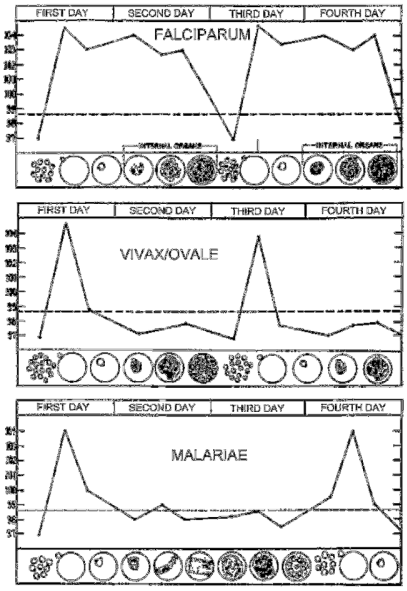

Figure 2: A schematicl pattern of temperature (in Farhenheit) in relation to blood-stage schizogony for the human malarial parasites. (Figure modified from Neva and Brown, 1994)

As long as the parasites reside within red blood cells the fever is relatively stable. However, as they exit the cells and enter the bloodstream, the immune system is alerted and initiates a response (Bate et al., 1988). This includes releasing cytokines capable

of inducing and regulating fever, resulting in an even further elevation of body temperature. Increased levels of body temperature are already associated with schizogony and the release/synthesis of several cytokines regulating this process can be induced or stimulated by LPS (lipopolysaccharide, a molecule extracted from the outer membrane of gram-negative bacteria, often used to induce inflammatory reactions (Wright et al., 1990). It is likely that parasite antigens released during schizogony have similar effects. This means that the period of fever rhythmicity depends on the developmental cycles, resulting in different cycles for species with different cycle lengths. . P. *falciparum*, *vivax*, and *ovale* all have 48 hour cycles, resulting in fever cycles of the same length. The developmental cycle of P. *malariae* takes approximately 72 hours, while *knowlesi* takes 24 hours to develop (Garcia, 2001). Why these developmental cycles are so varied and why they are always multiples of 24 hours is currently unknown.

An important cytokine pyretic (as well as antipyretic) effects that is released during schizogony is TNF (tumornecrosefactor). It is currently unknown what antigens are capable of inducing TNF release through antigen stimulated T-cells (Conti et al., 2004), though several studies have shown that exposure to S-antigen expressed in Plasmodium *falciparum* (also found in blood serum of malaria patients (Wilson et al., 1969) can lead to TNF expression. This antigen often associated with the (late) trophozoite stage and is also released during schizogony (Wilson & Bartholomew, 1975).

Development regulation

In 1929 Boyd showed that when the host was subjected to an inverted light-dark cycle, the parasite cycle was also inverted, and that schizogony occurred at midday rather than at night.. In 1976 Trager et al. showed that when kept in vitro, Plasmodium *falciparum* is completely arrhythmic. These experiments amongst others suggest that the parasite life-cycle is coupled to the circadian rhythm of the host. Using Plasmodium *knowlesi*, Hawking (1971) found that the number of gametocytes in the monkey's blood increased at night. A similar nocturnal rise in the number of gametocytes in the host's blood has since been recorded for several Plasmodium species. The cyclic and precise timing of gametocyte appearance in the vertebrate host to match the time of feeding of the mosquito was termed "the Hawking phenomenon" by Garnham and Powers (1974). Hawking hypothesized that the temperature cycle of the host affected and synchronized the parasites development. However in 1968 they concluded that this connection is somewhat unlikely based on several reasons including the fact that the hours of schizogony vary between species.

Melatonin

It appears that the rhythmicity of parasite development is caused by the parasites' ability to use its host's own circadian rhythm. In 1969, Arnold et al. showed that removal of the host's pineal gland resulted in desynchronisation of the intraerythrocitic development. Recent studies have found that melatonin (secreted by the pineal gland) is capable of synchronizing Plasmodium

development and that blocking melatonin receptors on the parasites' membrane abolishes this synchronization (Hotta, 2000). Melatonin is a hormone produced by the pineal gland. It's production is suppressed by (sun)light, resulting in a daily cycle in circulating melatonin levels, with low levels during the day and higher levels during nighttime (Lewy et al., 1980).

To enforce synchronization (and rhythmicity), melatonin needs to be able to provide a negative and/or positive effect on development at specific times. Several studies suggest that (high levels of) melatonin can have a positive effect on the maturation process, by inducing the release of Ca^{2+} during the trophozoite stage. Ca^{2+} has been linked to the maturation process and is essential for entering the schizont stage. (Hotta et al., 2000; Alves et al., 2010).

Melatonin binds to specific receptors on the parasite membrane and activates phospholipase C. This causes an increase in intracellular IP3. IP3 then causes a release of Ca^{2+} stored in the ER (endoplasmic reticulum). This effect is amplified by elevated cAMP levels resulting from this Ca^{2+} release, possibly resulting in the activation of pathways related to the maturation process. Whether this also results in a faster development is unknown, but it is possible. This would mean that melatonin has a positive effect on the development during a specific stage (trophozoite), during a specific time span (at night), something that could result in a more synchronized development. No negative effects of melatonin (or low levels of melatonin) are known, so far this is the only known mechanism in which melatonin can potentially influence development.

Although melatonin appears to be able to induce synchronization on its own, fever resulting from a multiple schizonts rupturing at the same time, might be able to aid synchronization: " *(I) When a large number of schizonts rupture at the same time, the resulting fever damages developing schizonts, thus temporarily preventing further parasite replication. (I!) The young progeny of the ruptured schizonts, however, can withstand the fever; so fever tends to synchronize the parasite population by selecting in favour of the progeny of the parasites that caused the fever. (3) When these progeny themselves undergo schizont rupture, 48 or 72 h later, fever recurs and reinforces the synchrony." (*Kwiatkowski, 1989)

Possible (evolutionary) pressures of rhythmicity

Hawking (1971) shares the view that fever helps synchronization. However, he focuses on the development of female and male gametocytes and their ability to infect mosquitos. When the gametocytes are mature, they are able to infect mosquitos for only a brief period (6 to 10 hours). This period of infectivity is generally timed at night, when mosquitos usually feed. He hypothesizes that this matching of the gametocytes infectivity to the mosquitos feeding pattern is the biological purpose of the rhythmicity found in the asexual cycle.

In 2011 O'Donnell and his colleagues studied the fitness costs of disrupted circadian rhythms for malaria parasites. They showed that a phase shift of +8 hours reduced both in-host replication (the asexual-cycle) and the production of transmission stages (gametocytes) by

approximately 50%. These costs are very likely to affect parasite survival and reproduction. Low in-host replication can leave the parasite vulnerable to clearance by the immune system on by treatment. A decreased between-host transmission could result in fewer infections of new hosts. Malaria parasites must optimize the trade-off between investment in in-host survival (the production of new merozoites that infect erythrocytes), and between host-transmission (the production of gametocytes capable of infecting mosquitos and infecting new hosts). O'Donnell et al. suggest that *"…there is a strong positive relationship with mosquito infectivity in terms of both the prevalence and intensity of mosquitos infected."* More broadly they suggest that circadian rhythms play an important role in the evolution of host-parasite interactions.

Kwiatkowski (1989) also mentions a possible advantage for the host. Febrile temperatures are not only directly damaging to mature intraerythrocytic parasites, they also enhance various immunological responses of the host (Dinarello et al., 1987). There is a possibility that the rhythmic fever actually inhibits parasite development. However, this would suggest that a disrupted circadian rhythm would result in a better survival and development of parasites. And as O'Donnell showed, this does not appear to be the case. With the data available, the rhythmic fever appears to be advantageous mainly to the parasite, even suggesting that the rhythmic development helps the parasite survive the onslaught of immune system attacks and maintain the infection. Other studies show that febrile temperatures do appear to inhibit parasite growth (in vitro) (Long et al. 2001), even in the earlier stages (as opposed to what Kwiatkowski found in 1989). Long and his colleagues conclude that: *"Long-lasting high body temperatures should lead to a faster reduction of parasites in patients with a high number of late-stage parasites."*

In short, although evidence is inconclusive, the parasites appear to gain more advantages from rhythmic fever than the host. It is, for example, suggested to help synchronize the developmental cycles. For the host, fever in general is helpful; however, it is not clear whether rhythmicity has any added benefits.

Possibilities for chronotherapy

Although the rhythmicity appears to have very little benefits for the hosts, it can potentially be useful in diagnosis or even treatment. When treating Plasmodium infections, speed and effectiveness are essential to prevent the development of severe malaria. Adapting the treatment to the stages the parasites are in can possibly enhance treatment while at the same time possibly reducing the frequency of drug administration. Especially when drug resistance is a real threat, this can be very helpful (Landau et al., 1991). Although most studies dealing with the possibility of chronotherapy test the now ineffective quinolone (derivative) treatments, it is possible that the same can be said of newer antimalarials. The fever rhythm can give an indication of the timing of schizogony and can possibly be used to time antimalarial medicine. Parasites in the ring stage are suggested to be more sensitive to artemisinin derivatives, and insusceptible to chloroquine treatment. Trophozoites on the other hand are more sensitive to

chloroquine treatment (Jiang et al., 1982). Pyrimethamine-sulfodoxine is more effective against merozoites found in capillaries. (Rieckman et al., 1987). *"Thus, treatment of malaria should be diversified according to the circumstances, and standardization of therapeutic schemes should take into account chronotherapeutical data. "* Landau et al., 1991

Fever treatment

Although I mentioned earlier that rhythmic fever can help parasites synchronize, fever itself (the elevation of body temperature, usually not rhythmic) when related to infections, appears to be something that helps fight the infection (Kluger, 1986). The same appears to be the case during malaria infections; febrile temperatures enhance the immune system and can inhibit parasite growth (Long et al., 2000). So in most cases, fever treatment might be unnecessary, however special cases wherein prolonged bouts of high fever pose a threat to the patients' health, may require treatment. Drugs like ibuprofen and paracetamol are often used to treat fever during malaria infections, especially in young children (Lell et al., 2001). Since the fever is rhythmic, a timed treatment could take the edge of potentially dangerous peaks, while not disturbing the less dangerous plateau.

A potential problem with chronotherapy based on developmental stages is that when mistimed, the treatment could potentially be counterproductive; less frequent and badly timed doses can result in less effective and slower treatment overall. This would be highly undesirable when speedy treatment is essential. Timed treatment may prove to be difficult in rural areas in the underdeveloped world where adequate clinical and laboratory supplies can be in short supply. At best, chronotherapy could be applied during hospital care where, hopefully, parasitaemia and paroxysmal periodicity can be monitored. Alghali (1991)

Conclusion

I set out to answer the following three questions: what causes the rhythmicity of both the developmental cycles and related fever? What are the benefits for the parasite and the host? And what are its implications for treatment?

The rhythmicity of fever during malaria infections appear to be coupled with the infecting parasites' asexual development cycles. Fever spikes occur as a result of schizogony in which mass release of parasites into the bloodstream can instigate the release of cytokines capable of inducing or elevating fever. It has been known for decades that plasmodium parasites use their hosts' circadian rhythm, though melatonin has only fairly recently been shown to be the guiding molecule. The mechanisms behind this regulation are mostly unknown, Melatonin induces Ca^{2+} release during the trophozoite stage, which is suggested to be involved in the maturation

process. Possibly, melatonin speeds up development during specific stages, causing schizogony to happen during the night.

Rhythmic and synchronized developmental cycles appear to have large advantages for infecting parasites. Several studies suggest that it allows the parasite to maintain the infection in the host and improve the chances of transfer to the mosquito.

Although it is likely that rhythmicity of fever is merely a side effect, induced by the release of approximately 30.000 mature parasites (which causes a rise in TNF-α and other cytokine levels), rhythmic fever during infection could provide an advantage for the plasmodium parasite. Since pre-schizont stages are far more sensitive to higher temperatures, it may help synchronization of the cycles by inhibiting parasites unable to synchronize. The advantages of this inhibiting are not clear, however it is suggested that unsynchronized parasites have both a lower in host-replication and production of gametocytes.

For the host, fever itself does enhance the immune systems response, as shown in several clinical and laboratory studies. However, it is unknown whether the rhythmicity provides any advantages for the hosts. Although certain stages (pre-schizont) of the parasite development are more sensitive to higher temperatures than others, the fever bouts are induced by a very specific event (schizogony). During this event the majority of parasites are no longer in a stage extra sensitive to higher temperatures. There is no evidence to suggest that merozoites released during schizogony are more susceptible to higher temperatures.

Unfortunately very little research has been done on the possibility of chronotherapy using more recent antimalarials. There are signs that different stages in Plasmodium development are more susceptible to specific antimalarials. A treatment with combined antimalarials timed to the most susceptible stages could enhance the overall treatment and potentially allow for less frequent doses. This can be very useful when resistance-development is an issue.

Although fever treatment is quite common, it can potentially prolong the infection. Febrile temperatures have been suggested to enhance the immune response, resulting in a better defense against the parasites. However, prolonged high febrile temperatures can result in brain damage and other complications. In cases where this is a threat, treatment with antipyretics is a viable option. Ibuprofen is known to reduce fever in malaria patients. As the fever peaks occur rhythmically in a majority of cases, timed fever treatment could potentially reduce this peak whilst not interfering with the lower plateau. Although this idea is not based on any previous studies, it could be interesting to test.

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