Abstract Published findings regarding the time structure of phagocytosis appear to be partly discordant. In addition, this feature has not yet been evaluated in rats, although the rat is an important biomodel used for haematological preclinical biomedical studies. Thus, we examined selected characteristics using rats in order to help elucidate the above-mentioned controversies and to provide further complete data on the haematology of this biomodel. The ingestion of foreign particles (HEMA) or large cells (yeast), the reduction of nitroblue tetrazolium salts (NBT) in rat circulating neutrophils and their migration were evaluated. We found circadian variations in the following characteristics of neutrophil phagocytosis: (i) phagocytic activity; the concentration of engulfed particles and phagocytic index decreases late in the day and peaks in the morning; (ii) NBT reduction; a rise being observed at noon and a fall in the evening. The acrophase for phagocytosis of larger yeast cells was earlier (small hours) than that of smaller HEMA particles (in the morning). Chemotactically oriented migration showed a significant increase in the afternoon, but we have not found a statistically significant fit for the cosine function of this characteristic. No circadian rhythm was present in spontaneous migration. Our findings support the opinion that changes in phagocytic characteristics are a part of the circadian system of the immune system in laboratory rats. By comparing our data with the literature it seems that discrepancies in the courses of the circadian rhythms can be at least partly caused by different laboratory procedures as well as by different acrophases for the various elements of phagocytosis.

Keywords Circadian rhythm · Circulating neutrophil · Phagocytosis · Rat

Abbreviations HEMA 2-hydroxyethylmethacrylate · MEM: minimal essential medium · NBT nitroblue tetrazolium salts · PBS phosphate buffered saline · ZAS zymosan-activated rabbit serum

Introduction

It is well known that various immunological and haematological characteristics vary under different physiological conditions. These changes create the time structure that supplements the spatial (anatomical, cytological) structure. One such phenomenon is the 24-h (circadian) rhythm, which has been described in both the count and the function of neutrophils. This circadian rhythm in the count of leucocytes, including neutrophils, in human and animals is well documented (Reinberg et al. 1977; Suzuki et al. 1977; Berger 1981; Haus 1996; Lilliehook 1997; Abrahamsen et al. 1999), whereas some data on the circadian variation in phagocytosis seem to be discordant or absent.

Phagocytosis starts by chemotactic movement of the cell and finishes after intracellular digestion of engulfed particles; several component of phagocytosis can therefore, be evaluated. The circadian structure of the neutrophil phagocytic index has been described in healthy men (Melchart et al. 1992) and in vivo in mice (Szabo et al. 1977). That of phagocytic activity has been documented for blood, spleen and peritoneal cells in mice (Knyszynski and Fischer 1981; Kurepa et al. 1992) and guinea pigs (Baciuc et al. 1988). The circadian rhythm of phagocytic index and activity has been shown in ring dove heterophils (Rodriguez et al. 1999). The circadian rhythm of superoxide anion by inflammatory neutrophils has been described in mice (Brigagao and
Colepicoio (1998) and that of neutrophil migration in men, mice (Bureau and Labrecque 1996) and cows (van Werven et al. 1996). Although some researchers have shown the circadian rhythm of several characteristics of neutrophil phagocytosis in men, mice (Bureau and Labrecque 1996) and guinea pigs (Baciul 1988), Bongrand and coworkers (1988) did not observe any circadian rhythms of phagocytic activity in human subjects.

Laboratory rats are an important biomodel for investigating various environmental effects on blood characteristics, including neutrophil functions and bio-rhythms (Berger 1987; Slapnickova and Berger 2002); however, no evaluations of the time structure of phagocytosis have been published in this species. Thus, we have examined selected characteristics of phagocytosis of rat neutrophils at different times.

**Materials and methods**

Experiments were performed on Wistar:H male rats in spring. They were housed under an artificial light regime LD 12/12 (light is on 7.00), humidity 40%–60% and 22–24°C; rats were allowed to adapt for 2 weeks before being used for this study at 8 weeks of age. Pelleted diet NOEH1 and water were available ad libitum. Peripheral blood was drawn, under slight ether narcosis, from the orbital plexus into heparin (35 IU/ml). Groups of six to nine animals per time point were investigated.

Yeast cells (large particles) or small hydrophilic particles were used in order to evaluate the phagocytic potential of circulating neutrophils. The mixture of heat-inactivated yeast cells of *Saccharomyces cerevisiae* (SC) and heparinised blood was incubated in polystyrene test tubes for 15 min at 37°C. Synthetic hydrophilic particles of 2-hydroxyethylmethacrylate (HEMA) copolymer (diameter 1.2 μm; Artim Prague) were suspended in phosphate buffer solution (pH 7.0–7.4) and incubated with heparinised blood in polystyrene test tubes for 60 min at 37°C (Berger 1988). We tested various incubation intervals and found that 15 min with yeast cells or 60 min with HEMA particles produced the most sensitive results. Blood smears were air dried and stained by the Pappenheim panoptic method; the number of particles per cell was counted using a Zeiss Axioplan 2 microscope with a Plan-Neofluar 100x/1.30 oil objective.

Results were expressed as mean ± SEM; the two-sided Mann–Whitney U test (p < 0.05) was used. The mesor (M), amplitude (A) and acrophase (Φ) were calculated from the cosine function Y(t) = M + A\(\cos(ωt + Φ)\), where ω = 360°/24 h, following fitting a time series of experimental data by the least squares method.

**Results**

Circadian variations in phagocytic activity of neutrophils were found using both yeast cells and HEMA particles. Phagocytic activity was observed to be lower during the light period (subjective night of nocturnal rats) and higher during the dark period, but the peak of phagocytosis of large cells occurred earlier – at noon, i.e. during the subjective day of rats (Fig. 1) – than that of small particles (in the morning; Table 3). The circadian rhythm of phagocytosing neutrophils with respect to smaller particles had a lower amplitude and higher mesor (Table 3).

The phagocytic index for small hydrophilic particles significantly decreased in the evening, whereas the peak for large yeast cells was found at noon (Fig. 2).