

Influence of Cyclophosphamide on the Haematological Profile of Laboratory Bred African Soft-furred Rats (*Mastomys natalensis*)

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Summary

The African soft-furred rat (*Mastomys natalensis*) has been shown to be a possible model for propagation of *Trypanosoma brucei gambiense*. This study aimed at determining the baseline biological reference values and reproductive data of a laboratory bred *Mastomys colony*, which was established at TRC. In addition, the effect of cyclophosphamide (an immunosuppressant) treatment (s) on the haematological profile was investigated. The mean gestation period was 23 days and the mean litter size was eight. At birth, the pups weighed 2.4±0.23 g and the weights increased to 78.0±10.6 g in males and 53.9±4.5 g in females by 90 days. The mean haematological values were significantly ($p<0.05$) higher in adults than juveniles. However, there was no statistical difference of haematological values between the sexes. Cyclophosphamide treatment caused a macrocytic hypochromic anaemia, which was noted 24 hours after treatment and was more severe in animals treated more than once. Thus, in studies involving a disease that causes anaemia, repeated cyclophosphamide treatment should be limited. Our study is a contribution to the clinical and biological characterization of the disease pattern in this preferred rodent model of *T. b. gambiense*.

Introduction

The search for a good rodent model of human African trypanosomiasis remains daunting, especially for the chronic disease caused by the *Trypanosoma brucei gambiense* parasite. The parasite rarely attains high parasitaemia in infected humans and experimental animals (Farah *et al.*, 2005; Maina *et al.*, 2003). Recent studies have suggested that the African soft-furred rat (*Mastomys natalensis*) is a probable candidate model for propagation of *T. b. gambiense* parasites (Maina *et al.*, 2003). This rodent is a multimammate rat found extensively in Africa (Coetzee,

1975). It has been used as a model to study a number of human and livestock diseases including lassa fever, filariasis and trypanosomiasis (Farah *et al.*, 2005; Ojok *et al.*, 2002; Murphy *et al.*, 1983).

We have recently established a *M. natalensis* colony at KARI-TRC with the aim using these animals for isolation and characterization of *T. b. gambiense* parasites from southern Sudan (Maina *et al.*, 2003). Before commencing the scientific studies on this species, it is important to determine the baseline values of the parameters expected to change during the disease progression. Thus, the current study was designed to determine the reproductive performance, bodyweight increase, and haematological values of *Mastomys natalensis* maintained in the colony. Cyclophosphamide (CY) is regularly used to immunosuppress rodents

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thereby allowing a high number of parasites to develop for use in pathogenesis and drug studies (Renoux *et al.*, 1980; Ngotho *et al.*, 2003). Due to CY's toxic effect on erythropoiesis, it is imperative to analyse the impact of CY treatment on the haematological profile and to determine the number of doses that will not threaten the life of the rodents.

Material and Methods

Animals

The initial *Mastomys* breeding stock was obtained from Livestock Research Institute (LIRI), Uganda and breeding was commenced at the TRC laboratory animal facility. The animals were maintained in Macrolone cages with the following dimensions: 14 cm width, 30 cm length, and 15 cm depth. The animals were fed on rat/mice pellets (Mice pencils®, Unga Feeds, Nakuru, Kenya), fresh vegetables, carrots, and groundnuts. Water was provided *ad libitum* and the ambient temperature ranged between 20 and 25 °C. The bedding used was wood shavings (Tim Sales, Nairobi, Kenya), which was changed twice a week.

In each cage, a male was kept with two females. Inbreeding (brother x sister mating) was chosen as the breeding programme. To determine the breeding performance the gestation period, littering period, and litter size, six breeders were randomly selected and assessed for a period of one year. The body weights of the offspring were recorded at birth (pups), 40 days (juveniles around puberty), and 90 days (adults).

Determination of the baseline haematological reference values

To determine the reference ranges of the haematological values of the juvenile and adult *Mastomys*, blood (100µl) was collected from the tail vein into Eppendorf tubes containing EDTA. Detailed haematology analysis was conducted using an automated haematology analyser (Coulter Beckman, Miami, USA). A prior pilot study showed that the haematological values for a given

sample could be reproduced with a variation ranging between 1-2% for the erythrocyte and white blood cell (WBC) counts, and 3-5% for the platelet values. For the erythrocyte indices, the parameters evaluated included: total red blood cell (RBC) count, haemoglobin (HB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Total platelets (PLT) and white blood cell counts were also performed. A descriptive analysis of the variables was undertaken using Statsview® statistical package.

Effect of cyclophosphamide on haematology

Twenty-five *Mastomys* were used for this study. The rodents were divided into five groups consisting of five adult females each. The animals were injected intraperitoneally with cyclophosphamide monohydrate (Fluka Chemie, Steinheim, Switzerland) at 200 mg/kg bw and was repeated every 10 days as shown in Table 1. Five adult females were used as controls. Body weight was measured every seven days, while blood (100µl) was collected one day before and after treatment. Blood was sampled on -1, 1, 5, 9, 11, 15, 19, 21, 25, 29, 31, 35, 39, 41 days after treatment. The blood was analysed for different haematological values as described above. At the end of the experimental period, all rodents were euthanised by inhalation of 95% carbon dioxide.

Table 1. The treatment and sampling points of the experimental *Mastomys natalensis*

Groups	Days of CY treatments
I	0,10
II	0, 10, 20
III	0,10, 20, 30
IV	0, 10, 20, 30, 40
V (Control)	None
Key: CY = cyclophosphamide	

Ethical review process

All protocols and procedures used were reviewed and approved by the KARI-TRC Institutional Animal Care and Use Committee.

Results*Breeding pattern*

In the wild as well as in captivity *Mastomys* breed all the year round. The average gestation period recorded was 23 days (range of 19 to 24 days, day-1 being the day a vaginal plug was observed) and the young were weaned 21 days after birth. The average number of pups was 8 (range 4-12). The third to fifth litters had the highest number of pups (range 10-12). Due to a decline in litter size, the breeders were euthanised after the eighth litter was weaned.

Body Weight

The mean average body weights are shown in Table 2. At each age group, males were significantly ($p<0.05$) heavier than females.

Haematology reference ranges

There was no significant difference between male and female values and thus the results for both sexes were pooled. The adult haematological parameters were higher than those of the juvenile animals, except MCHC (Table 3). The platelets were also significantly ($p<0.05$) higher in adults than in juveniles.

Health monitoring

Since the colony was established, papillomatosis was the most common disease with a yearly preva-

Table 2. Weight changes in normal *Mastomys natalensis*

Category	Number	Mean±SD	Range
Pups	65	2.4±0.2	1.7-2.8
Juvenile males (40 days old)	30	34.1±4.0	27.0-43.0
Juvenile females (40 days old)	30	29.0±2.1	25.0-32.0
Adult male (90 days old)	30	78.0±10.6	61.0-93.0
Adult females (90 days old)	30	53.9±4.5	45.0-63.0
SD = Standard deviation			

Table 3. Normal haematological values of *Mastomys natalensis* grouped according to age

Hematology parameter	Juvenile		Adults	
	Mean±SD	Range	Mean±SD	Range
RBC ($\times 10^6/\mu\text{l}$)	7.5 ±0.5	6.2 – 8.7	7.9±0.8	5.5-9.5
HB (g/dl)	14.2± 1.0	11.5 – 16.6	14.8± 1.3	10.6 – 18.3
HCT (%)	38.3± 2.3	31.7 – 43.6	44.5± 6.1	34.4 – 65.0
MCV (fl)	51.6± 1.8	49.0 – 59.1	55.1± 4.0	46.0 – 64.8
MCH (pg)	19.1± 0.5	18.0 – 20.2	20.1± 4.3	17.3 – 34.8
MCHC (g/dl)	37.0± 0.9	35.4 – 39.0	36.9± 7.8	30.8 – 58.4
PLT ($\times 10^3/\mu\text{l}$)	460.0± 103.8	300.0 – 813.0	751.0± 257.0	186.0 – 1272.0
WBC ($\times 10^3/\mu\text{l}$)	10.8 ±1.9	6.8 – 15.4	11.9 ±2.6	7.5 – 20.0
Key: SD = standard deviation, other abbreviation are as indicated in the materials and methods				

lence of 8%. This disease was observed more commonly among the aged breeders than among the younger animals. There was no mortality associated with the condition and in all cases the affected animals were euthanised. A few instances of mange infestations were noted and effectively treated intraperitoneally with Ivermectin 1% injectable solution (Ivermectin®, Anupco, Suffolk, England) at a dosage of 0.2 mg/kg bw.

Effect of Cyclophosphamide on haematology Red Blood Cells

In all the treated groups, the RBC decreased from counts above 8×10^6 to counts less than $4 \times 10^6/\mu\text{l}$ within 24 hours after treatment. The RBC counts in the rats that were treated only once had a higher recovery than the other groups although they never attained the pre-treatment levels. The rate of decrease was more severe after the first CY administration than after subsequent doses. The most marked erythrocyte decrease (60%) was observed at 34 days post-treatment in the group treated with three doses of CY.

Haemoglobin

The decrease in HGB was more severe after the first and second CY administration, while the recovery was more evident in group I, 9 days post treatment. Thereafter, the increase was inconsistent and characterized by fluctuations.

Haematocrit

A significant decrease was always observed after every CY administration, and this was always followed by a temporary increase within 2-3 days. The lowest HCT was observed at 25 and 35 days post-treatment. Groups I and II had a significant recovery and achieved pre-infection levels, although fluctuations were observed.

Mean corpuscular volume

There was a consistent increase in MCV between days 1 to 10 post-treatment in all the groups (56.0 to 69.2 fl). CY administration on days 10, 20, 30

caused a slight decrease in MCV, which was followed by significant rise.

Mean corpuscular haemoglobin concentration

A decrease in levels from 32.6 to 30.1 g/dl was observed between days 0 and 10 post-treatment, after which the levels fluctuated being higher in groups II and IV than in the other groups. CY administration was always followed by slight decline the day after. Nevertheless, peaks were observed in between the treatments.

Discussion

Findings from the present study confirm that *M. natalensis* breed and thrive under standard rodent laboratory conditions. As reported by Jackson and Van Aarde (2002), males exhibited a faster growth rate than the females. The breeding pattern was found to be similar to that reported by Coetzee (1975) and Davis (1963). The average litter size recorded in the present study was in good agreement with that reported in colonies in the wild e.g. in Uganda: 12.1 (Delany & Neal, 1969), Malawi: 11 (Hannes, 1965), and South Africa: 10 (Coetzee, 1975), and similar to other laboratory-bred colonies (Davis, 1963). Both *M. natalensis* and *M. coucha* respond to sub-optimal diet by reducing litter size and litter mass (Coetzee, 1975; Jackson & Van Aarde, 2004) and the data from the present study indicate that the laboratory conditions provided met with the animals' needs. The reduction in litter size with age was expected and well-known from laboratory mice and rats, and has also been reported in other rodents (Meserve & Bouleng, 1987).

There are no reference haematological values of *M. natalensis* in the literature. Since the values can be influenced by factors such as environmental conditions, breeding system, feeding, and lineage the results may not be representative for other colonies of this species. Indeed, every laboratory facility has been encouraged to establish its own reference values (Ringler & Dabich, 1979). Several observa-

tions can be drawn from our study. The lower mean haematological values observed in juveniles were not unexpected and is probably a reflection of the haematopoietic system not yet being fully developed. The absence of significant differences between female and male haematological values confirms the findings reported in some studies (Kojima *et al.*, 1999), although others have reported that male rodents have higher levels of red cell indices than do females (Teixeira, *et al.*, 2000). The mean MCHC values (37-36.9g/dl) were higher than those reported for the common laboratory rat (*Rattus norvegicus*) (33 – 34 g/dl) (Teixeria *et al.*, 2000). For the African giant rat (*Cricetomys gambianus*, Waterhouse) the RBC ($5.50-6.19 \times 10^6/\mu\text{l}$), MCHC (23.4-30.8 g/dl) and WBC ($7.1-8.6 \times 10^3/\mu\text{l}$) values were lower (Olayemi *et al.*, 2001) than those reported for *Mastomys* in the current study. However, the mean PCV (47.7-50.5) and MCV (80.6-96.5 fl) for the African giant rat were higher (Olayemi *et al.*, 2001) than those of *Mastomys* reported in our study.

Cyclophosphamide (CY) is an alkylating agent, which prevents cell division primarily by cross-linking DNA strands (Chabner & Myers, 1989). Its inhibitory effects on rapidly dividing cells makes it an effective drug against some forms of cancer and certain immune mediated diseases (Chabner & Myers, 1989). The immunosuppressive effect of the drug on laboratory animals makes these more vulnerable to most experimental infections and may mediate changes from sub-clinical infection to disease. In trypanosomosis studies, rodents are routinely treated with CY before trypanosome inoculation, and this treatment increases the level of parasitaemia (Jones, 1986). Due to the slow growth rate of *T. b. gambiense*, multiple doses of CY are required to immunosuppress the recipient animals (Ngotho *et al.*, 2003). However, the biological effects of the treatment on the animals have not been clearly defined and characterised. In this study, macrocytic hypochromic anaemia was observed in animals that were treated with CY. The anaemia occurred 24 hours after treatment and was

followed by a slight and gradual recovery at nine days post-treatment. Significant decreases and a slow recovery rate of most haematology values were observed in *Mastomys* treated more than once with CY and very pronounced in those treated three or more times. The haematological values rarely achieved pre-treatment values and were characterized by fluctuations. The change in MCV indicates that after the first CY dose, the precursor red cell reacts immediately by producing relatively large and immature RBC, but subsequent CY doses cause minimal changes in RBC size. Considering that *T. b. gambiense* also causes anaemia (Emeribe & Anosa, 1991), it was expected that animals infected and subsequently subjected to CY administration would develop severe anaemia leading to high mortalities. Indeed, during the development of an immunosuppression protocol for effective *T. b. gambiense* recovery at our laboratory, repeated inoculation of *Mastomys* with CY resulted in some mortalities (Ngotho *et al.*, 2003). Thus, based on the current findings and those of previous studies we recommend that treatment not be repeated more than three times.

In conclusion, our study has contributed to the growing information on reproductive data for *Mastomys*. More importantly, haematological reference values, and the effect of cyclophosphamide on the haematological profile of laboratory-bred *M. natalensis*, have been scrutinised. Further studies on the suitability of this animal as a model for immunological and pathological studies of experimental *T. b. gambiense* infection are in progress at our laboratory.

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