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The effect of L-thyroxine on the anaemia response in *Trypanosoma congolense* infected rabbits

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Abstract

The development of anaemia is a major pathological manifestation in chronic trypanosomosis. The anaemia in African trypanosomosis coincides with a marked decrease in plasma concentration of both thyroxine (T_4) and 3,5,3′ triiodothyronine (T_3). To evaluate the effect of trypanosome-induced hypothyroidism on the development of anaemia, sexually mature white New Zealand rabbits were used. Three groups were set up, each of ten rabbits: one group was infected with *Trypanosoma congolense*; the second group was infected but given replacement doses of thyroxine (treated); the third group was not infected. Small volumes of blood were collected for the determination of parasitaemia and packed cell volume (PCV). The concentrations of T_3 and T_4 were measured in plasma by radioimmunoassay. The decrease in PCV correlated closely ($y = -0.38x + 15.2$; $r = 0.82$, $P = 0.001$) with the intensity and duration of parasitaemia. The critical PCV value was 0.15 l l^{-1} with a peak parasitaemia of approximately 5×10^6 trypanosomes ml^{-1} of blood. There was a significant correlation between the plasma T_3 and PCV ($y = 0.049x + 0.57$; $r = 0.66$, $P = 0.020$). There was also a good positive correlation between T_4 and PCV ($y = 14.5 + 3.03$; $r = 0.95$, $P < 0.001$) in the infected untreated group. The PCV levels were significantly different among the three groups of animals ($P < 0.05$). The infected–treated animals sustained longer periods of infection than the infected and untreated ones. The sustained physiological level of bioactive thyroid hormones T_3 and T_4 significantly arrested the decline in PCV as the disease progressed. The hormonal treatment thus enhanced the survival of the animals infected with *Trypanosoma congolense*.

Keywords: *Trypanosoma congolense*; Rabbit; Thyroxine; Triiodothyronine; Anaemia

1. Introduction

Among the most pronounced physiological effects attributed to chronic trypanosomosis is the depression in plasma thyroxine level (Mutayoba et al., 1988; Lomo et al., 1993). The

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fall in thyroid hormone level coincides with the decrease in packed red cell volume (PCV) which is an indicator of anaemia (Murray and Dexter, 1988). Although previous work indicates that parasitic or chemically induced anaemia may be resolved by testosterone or erythropoietin administration (Haurani and Green, 1967; Zucker et al., 1974), no report is available on the response of anaemia to a sustained euthyroid condition.

The thyroid hormones regulate the biogenesis of mitochondria by stimulating protein synthesis as well as activating energy-transducing molecules such as AMP and the Na^+/K^+ ATPase system (Edelman, 1975; Nelson et al., 1984). In a preceding paper, Lomo et al. (1993) reported impaired mitochondrial respiratory function attributed to severely depressed thyroid hormone concentration. The extramitochondrial influence of the thyroid hormone in the activation of nuclear DNA transcription with subsequent RNA synthesis was previously described by Barletta et al. (1973).

The foregoing primary biochemical events are likely to influence the severity of the anaemia through precursor synthesis, and hence the survival chances of the infected subjects. The thyroid hormones regulate growth and development in vertebrates. The erythropoietic response of the bone marrow cells may be dependent on exposure of the haemopoietic progenitor cells to a euthyroid condition. The decreased physiological levels of thyroxine may indeed be part of the aetiology of the anaemia produced by severe trypanosomosis. This study was therefore designed to evaluate the effect of thyroxine replacement therapy on parasite-induced anaemia in rabbits experimentally infected with *Trypanosoma congolense*.

2. Materials and methods

2.1. Animals

Thirty sexually mature male New Zealand white rabbits (2.8–3.0 kg) were used in this study. The animals were divided into three groups; infected (ten rabbits), infected but treated with thyroxine (ten rabbits) and normal controls (ten rabbits). The animals, which were housed in individual cages (35 cm × 45 cm × 30 cm) within a well-ventilated room, were maintained under standardized conditions of light (12 h light, 12 h darkness) and temperature (25°C). Rabbit pellets, green vegetables and water were given ad libitum.

The 20 experimental animals were each infected through the lateral ear vein with approximately 1×10^4 of a *T. congolense* stabilate (Clone IL-3000), which was obtained from the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi. Using ethylene diamine tetra-acetic acid (EDTA; pH 7.4) as anticoagulant, blood samples (0.3 ml) were collected through the lateral ear vein twice a week. The parasitaemia and PCV were determined as previously described by Paris et al. (1982).

2.2. Hormonal treatment of the animals

Thyroxine replacement therapy was initiated upon infection and appearance of the parasites in the blood—usually 1 week after infection. L-Thyroxine marketed by Glaxo East Africa Ltd. as 'Eltroxine 100 Glaxo' was used in the experiment. Plasma T_3 and T_4 levels

were determined twice weekly in all the animals. The physiological levels of the thyroid hormones were maintained in the infected–treated group by i.p. injection of 50 μg of T_4 per 100 g of body weight delivered in phosphate-buffered saline (pH 7.4). The experiment included controls which were infected as well as non-infected animals, none of which were subjected to hormonal therapy. However, the control animals received vehicle (phosphate-buffered saline, pH 7.4) only.

2.3. Radioimmunoassay

Plasma T_3 and T_4 were measured by a solid-phase ^{125}I-labelled radioimmunoassay kit (DPC, Los Angeles, CA, USA). The intra-assay coefficient of variation (CV) for T_3 was 4.6% at 2.16 nmol l^{-1} and 6.2% at 41.5 nmol l^{-1} for T_4 ($n=20$). The inter-assay CV for T_3 was 11.4% at 2.58 nmol l^{-1} and 7.5% at 75.1 nmol l^{-1} for T_4 ($n=15$). The assay sensitivity (minimum detectable dose), calculated as the concentration which is 2 SD above the zero standard, was 3.79 nmol l^{-1} ($n=20$).

2.4. Statistical analysis

The results are expressed as mean $\pm$ standard error of the means (SEM). The data were subjected to statistical analyses using analysis of variance (ANOVA), and the correlation between groups was determined by linear regression analysis (Sokal and Rohlf, 1981). The significance level of the tests was taken as $P \leqslant 0.05$.

3. Results

The normal (non-infected) animals showed a PCV range between 0.38 and 0.46 l l^{-1} with a mean of 0.41 ± 0.02 l l^{-1}. The mean weekly T_3 and T_4 levels were 2.67 ± 0.02 nmol l^{-1} and 76.2 ± 1.4 nmol l^{-1}, respectively. The variation in thyroid hormone levels between individual rabbits within the non-infected group was not significant.

A drastic fall was observed from pre-infection values of 2.77 ± 0.04 to 2.15 ± 0.07 nmol l^{-1} for T_3 and from 85.0 ± 4.5 to 42.1 ± 3.9 nmol l^{-1} for T_4, within the first 3 weeks of infection, as shown in Fig. 1(a). This correlates with an increase in parasitaemia to about 5×10^4 trypanosomes ml^{-1} of blood in the third and fourth week. The general trend of decline in T_3 and T_4 continued, reaching a minimum value of 1.23 ± 0.22 nmol l^{-1} and 18.4 ± 3.5 nmol l^{-1}, respectively, in the tenth week, with a corresponding parasitaemia of about 5×10^6 trypanosomes ml^{-1}.

The effect of trypanosome infection on the PCV is shown in Fig. 2(a). The PCV was determined a week prior to infection. This provided a baseline PCV value besides those of the normal controls. A progressive decline in PCV was observed throughout the 10 weeks of infection, reaching a minimum value of 0.12 ± 0.03 l l^{-1}. The gradual decline in PCV of the infected animals was positively correlated with the rise in parasitaemia. The equation for the regression line was $y = -0.37x + 15.2$ ($r=0.82$, $P=0.001$). The difference in variance of the means of PCV were significant between non-infected and infected–untreated

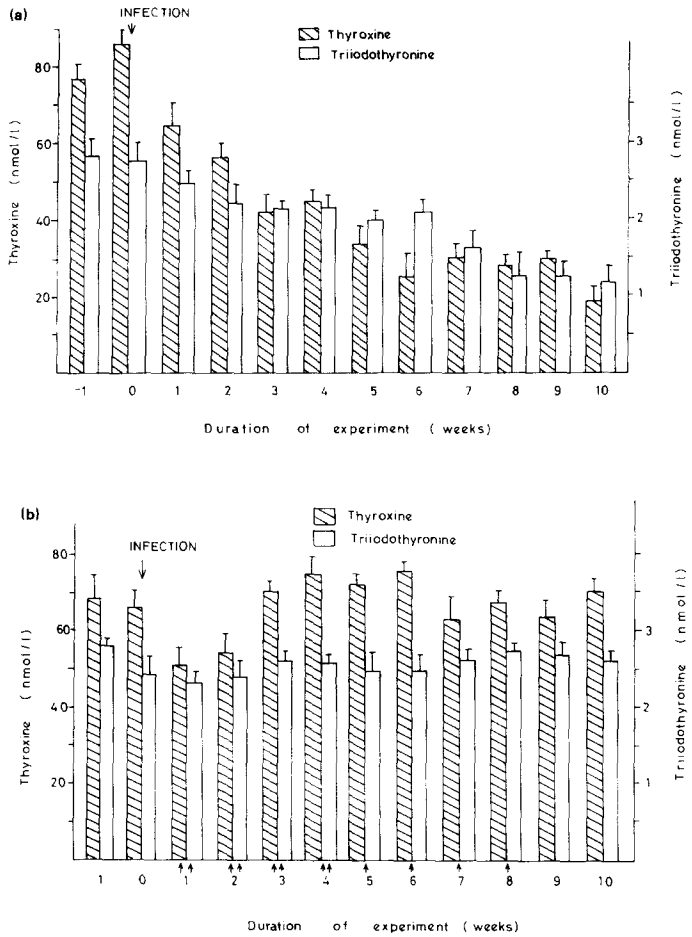

Fig. 1. (a) Effect of *Trypanosoma congolense* infection and plasma concentration of T_3 and T_4 in the untreated group. The results are expressed as mean ± SEM; $n = 10$ in Weeks 1–6, $n = 8$ in Weeks 7 and 8, $n = 5$ in Week 9, $n = 4$ in Week 10. (b) Plasma concentration of T_3 and T_4 in the animals receiving thyroxine treatment following *Trypanosoma congolense* infection. The results are expressed as mean ± SEM; $n = 10$ in Weeks 1–8, $n = 9$ in Week 9, $n = 8$ in Week 10. ↑ ↑, Daily thyroxine administration; ↑, thyroxine injection twice weekly.

($P < 0.001$), non-infected and infected–treated ($P < 0.001$), and infected–untreated and infected–treated ($P < 0.05$) group.

The plasma concentrations of T_3 and T_4 during thyroxine treatment of the experimentally infected rabbits are shown in Fig. 1(b). The replacement therapy was started a week post-infection, when all the animals had shown an initial wave of parasitaemia. A remarkable increase in T_4 level (73.33 ± 2.5 nmol l^{-1}) as a result of hormonal treatment was detected in the third week. The physiological levels of the thyroid hormones were maintained throughout the 10 weeks of experiment. The correlation between T_3 and PCV ($y = 0.049x + 0.57$; $r = 0.66$, $P < 0.020$), T_4 and PCV ($y = 14.5x + 3.03$; $r = 0.95$, $P \leqslant 0.001$) were significant in the infected–untreated group. There was no significant dif-

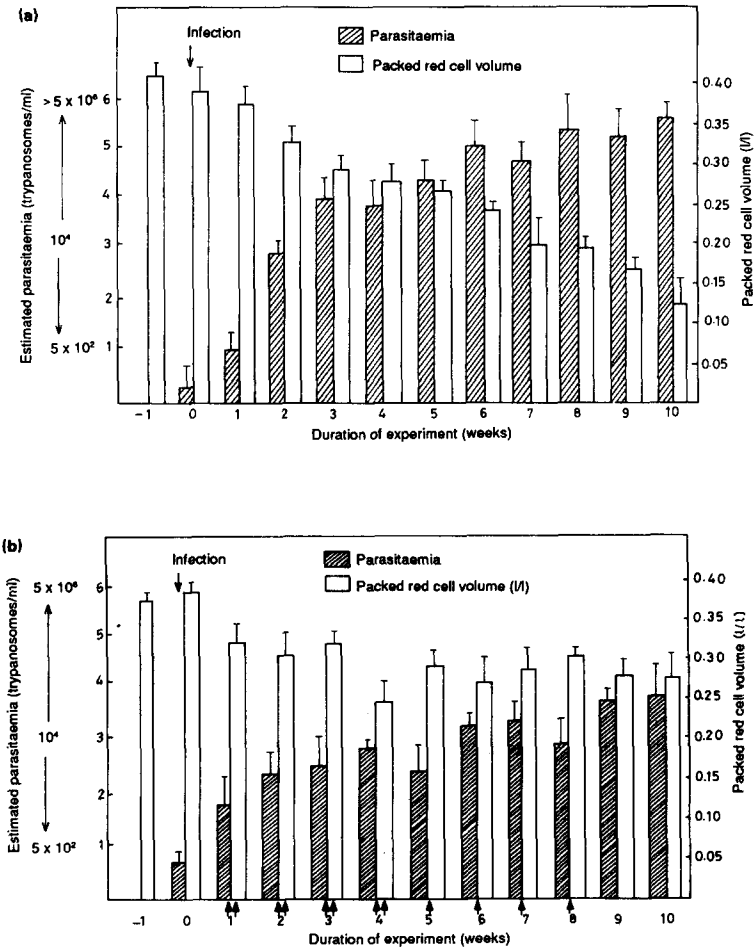

Fig. 2. (a) Parasitaemia score and packed red cell volume following *Trypanosoma congolense* infection in the untreated group. The results are expressed as mean ± SEM. The *n* values are as in Fig. 1(a). (b) Effect of thyroxine replacement on parasitaemia score and packed red cell volume following *Trypanosoma congolense* infection. The results are expressed as mean ± SEM. The *n* values are as in Fig. 1(b). ↑↑, Daily dose of thyroxine; ↑, thyroxine administration twice weekly.

ference in the correlation coefficient of PCV and T_3 or T_4, in either infected–treated or normal control group.

The effect of sustained thyroid hormone level on PCV is shown in Fig. 2(b). The increase in parasitaemia between Weeks 0 and 2 caused a notable decrease in PCV. Further increase in parasitaemia did not cause a consistent decrease in PCV. The infected thyroxine-treated animals maintained a mean PCV value of $0.28 \pm 0.02\ 1\ l^{-1}$ between Weeks 4 and 10. However, the PCV did not return to normal haematological values ($0.41 \pm 0.02\ l\ l^{-1}$) even in animals that had achieved complete recovery with no detectable parasites in their blood. The infected–untreated group attained a significantly ($P < 0.01$) higher parasitaemia level compared with the infected–untreated subjects.

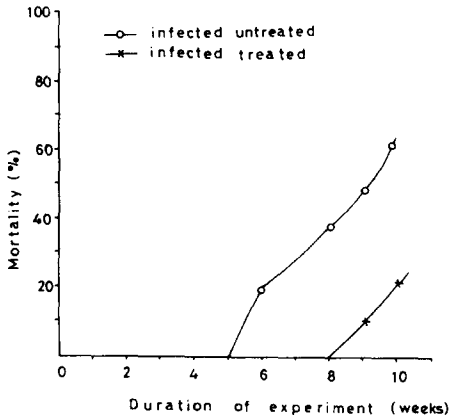

Fig. 3. Effect of thyroxine administration on percentage mortality of trypanosome-infected rabbits. The mortality is expressed as a fraction of the dead over the total number of animals used in the individual experiments.

A comparison of percentage mortality of the infected rabbits that received thyroxine replacement (treated) with those infected but not subjected to hormonal treatment (untreated) is shown in Fig. 3. The infected–untreated group succumbed to the parasitic infection from the sixth week of infection, whereas the initial death among the infected–treated subjects was observed from the ninth week of infection. Thus the hormonal treatment enabled the infected animals to withstand a relatively longer period of infection. None of the uninfected rabbits died in the course of the experiment.

4. Discussion

The essential finding of the present study is the demonstration that thyroid hormone replacement moderates the severity of trypanosome-induced anaemia and thus enhances the survival chances of the infected subjects. Anaemia remains the major pathological manifestation in chronic trypanosomosis (Murray and Dexter, 1988). The decrease in plasma concentration of 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) following trypanosome infection has been previously reported (Mutayoba et al., 1988; Lomo et al., 1993).

The packed red cell volume (PCV) is used as an index of anaemia and measures the rates at which red cells are added to and withdrawn from circulation by synthesis and breakdown, respectively. By simultaneous measurement of plasma T_3 and T_4 levels during the infection, it was possible to investigate direct and/or indirect influence of these thyroid hormones on the development of anaemia as measured by PCV. In cattle infected with *T. congolense*, increased red cell breakdown commences with the development of parasitaemia (Preston et al., 1982). The presence of the parasite is the basis of the progress of the anaemia. Once the parasite has been eliminated from the blood then there is a return to normal haematological values (Holmes and Jennings, 1976). However, our findings in *T. congolense*-infected rabbits show that low PCV values persist even in cases where the animal's immune system has successfully eliminated the parasite from the bloodstream.

This suggests that several factors, acting singly or in concert, besides the physical damage to the red blood cells by the living trypanosomes, cause the trypanosome-induced anaemia (Banks, 1980).

After the first peak of parasitaemia, the antibody response results in a major trypanolytic crisis, of which several subsequently occur with each successive parasitaemia peak. These crises lead to formation of antigen–antibody complexes (Murray and Dexter, 1988) and probably the release of a whole range of biologically active factors known to be present in trypanosomes (Tizard et al., 1978). The antigen–antibody complex may activate the complement system, which could in turn lead to the destruction of the red blood cells of the host. The significantly ($P < 0.001$) lower level of parasitaemia attained in the infected–treated group relative to the infected–untreated group suggests that the hormonal treatment probably enhanced the ability of the infected subjects to fight off the parasites. This may also account for the significantly ($P < 0.05$) lower degree of anaemia observed in the infected–treated group.

Perhaps the most interesting feature in our observations is the close correlation between the decline in thyroid hormone levels and the decrease in PCV as the disease progresses. This decrease in thyroid hormone level may be implicated in the induction of anaemia in the light of the pronounced physiological effects of these hormones on respiratory metabolism (Lomo et al., 1993). Thyroid hormones influence thermogenesis and biogenesis of the mitochondria (Nelson et al., 1984). T_3 in particular has an extramitochondrial effect of activating nuclear DNA transcription with subsequent RNA synthesis (Barletta et al., 1973). It therefore appears reasonable to suggest that under trypanosome-induced hypothyroid condition, the biochemical effects together with secondary events such as activation of energy-transducing molecules such as AMP and the Na^+/K^+ ATPase system may influence the erythropoetic activity (Edelman, 1975).

Thyroxine replacement prevented the trypanosome-induced anaemia to some degree (Fig. 2(a)). It prolonged the survival period of infected–treated rabbits (Fig. 3). The rational interpretation of these data is that sustained physiological levels of thyroid hormones maintained the synthesis rate of red blood cells so that further development of anaemia was more from enhanced breakdown rather than diminished synthesis. However, this hypothesis could only be verified from experiments that measure the rate of red blood cell synthesis. These results corroborate the previous observations of Haurani and Green (1967) and Zucker et al. (1974), who reported that parasitic or chemically induced anaemia may be reduced by hormonal administration. The hormones testosterone and erythropoietin are thought to have direct action on haemopoietic progenitor cells besides being capable of directly or indirectly releasing iron from the reticulo-endothelial system. Thyroid hormones probably enhance erythropoietic activity through a similar mechanism in addition to their known biochemical effects.

Exposure in bone marrow of nucleated erythroid precursors to the thyroid hormone deficient state may thus explain the poor clinical response to trypanocidal drug therapy in animals with long-standing infection. Thyroid hormone replacement may therefore have a therapeutic value in trypanosomosis.

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