

University of Glasgow Veterinary School

Report

Plasma nitric oxide levels in *T. congolense* infected Scottish Blackface sheep fed a normal or a urea-supplemented diet

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Appendix II

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The interaction between nutrition and genetic resistance to trypanosomiasis in trypanotolerant livestock.

REPORT

Plasma nitric oxide levels in *T.congolense* infected Scottish Blackface sheep fed a normal or a urea-supplemented diet

Introduction

Macrophages have been found to produce nitric oxide during trypanosome infections. Nitric oxide has been related to immunosuppression through the suppression of parasite-antigen-specific T-cell proliferative responses (Schleifer and Mansfield, 1993) and to cytostatic effects on trypanosomes in vitro (Mabbott *et al.*, 1994; Vincendeau *et al.*, 1991, 1992; Sternberg *et al.*, 1994). In vivo, however, haemoglobin scavenges nitric oxide and is unlikely to have a significant cytostatic effect on trypanosomes (Mabbott *et al.*, 1994; Sternberg *et al.*, 1994).

This experiment was carried out to investigate whether nitrate levels, one of the stable end-products of nitric oxide, were raised during a *Trypanosoma congolense* infection of Scottish Blackface lambs fed a normal or a urea supplemented diet and whether these nitrate levels were related to the number of trypanosomes found.

Materials and methods

Experimental design

Twelve pairs of male, twin Scottish Blackface lambs were selected and divided into two groups of six pairs (N and U). The lambs were approximately 8 months of age. All animals received 428 grams grass hay dry matter (DM) per day. One group of six pairs (N) were offered an additional amount of 395 grams DM of barley grain and the other group (U) 400 grams DM of barley grain plus urea. The diets resulted in estimated crude protein levels of 86 and 123 grams per day for normal and urea supplemented diets respectively. Energy levels in both diets were very similar. Two weeks after the start of the experiment one animal of each pair was infected (I) with *T.congolense* and the other animal used as a control (C).

Infection

Two weeks after the experiment started the group I lambs was infected with *T.congolense* 1180 (GRVPS 57/6) (Nantulya *et al.*, 1984). The trypanosomes were obtained from irradiated mice during the first rising parasitaemia. Each lamb was inoculated intravenously with 5×10^5 trypanosomes in 3 to 4 ml phosphate buffered saline (PBS) (containing 1.5% glucose).

Measurements

On Mondays and Thursdays 5 ml of blood was collected into tubes containing lithium heparin for plasma nitrate measurements. The samples were collected from the jugular vein using 20 gauge needles between 7:30 and 8:30 a.m., just before the morning feeding.

Parasitaemias were measured using the buffy coat method (Murray *et al.*, 1977; Paris *et al.*, 1982).

Statistical analysis

The plasma nitrate concentrations were subjected to statistical analysis using a randomised block design with each block consisting of a pair of lambs (one I, one PC). Mean effect over time was calculated and subjected to split plot analysis of variance with consideration of variation between treatments, between animals within treatments and interaction between treatments. The relationship between parasitaemia and plasma nitrate concentrations was investigated using the Kruskal Wallis test.

Results

A slight difference in plasma nitrate levels (Table I) was found before infection between the two diet groups with the animals on the normal diet showing the higher levels ($p < .05$). The *T.congolense* infection caused a rise in plasma nitrate concentrations (Figure 1) in both dietary groups. The rise was similar in both groups.

No relationship was found between parasitaemia level and plasma nitrate concentration. However, on days when the highest number of parasites were found plasma nitrate concentrations were on average 10 points higher (Table II).

Discussion

Nitric oxide produced by macrophages during trypanosome infections has on the one hand been related to immunosuppression in the host and on the other hand found to be cytostatic to trypanosomes *in vitro*. More recent reports, however, suggest that the cytostatic effects of nitric oxide to trypanosomes does occur *in vivo*, possibly due to the capture of nitric oxide by haemoglobin. In fact, inhibition of nitric oxide synthesis has lead to reduced parasitaemias in murine *Trypanosoma brucei* infections which may be a consequence of the inhibition of the

immunosuppressive effects of nitric oxide (Mabbott *et al.*, 1994; Vincendeau *et al.*, 1991, 1992; Sternberg *et al.*, 1994).

The nitric oxide scavenging activity of haemoglobin, however, appeared not to prevent a significant increase in plasma nitrate concentration during the trypanosome infection in this experiment. It is, however, difficult to determine whether the nitrate levels were high enough to have a significant cytostatic effect on the trypanosomes. Plasma nitrate levels were highest when the number of parasites found was very high. It is likely that the high number of parasites induced the macrophages to produce nitric oxide.

Excessive production of nitric oxide has been found to lead to pathological effects such as acute and chronic inflammation (Ianaro *et al.*, 1994) and arthritis (McCartney-Francis *et al.*, 1993).

One of the properties of nitric oxide is that it reacts with superoxide, also produced by activated phagocytes (Bellavite, 1988), to produce peroxynitrite. Peroxynitrite has been found to cause aggregation of human platelets (Moro *et al.*, 1994). The sheep used in this experiment showed a decrease in numbers of platelets (See previous report) which may have been caused by aggregation.

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Table I Mean plasma nitrate concentration (μM) of *T.congolense* infected (I) sheep and their respective controls (C) fed a normal (N) or a urea supplemented (U) diet during pre- (day -9 - 0) and post-infection (day 14 - 49)

Group	Nitrate concentration (μM)	
	Period	
	pre	post
	14.0	17.3
NC	13.0	10.3
Pooled SE	0.8	1.4
UI	11.5	15.0
	10.2	8.6
Pooled SE	0.8	1.2
Diet effect	*	ns
Infection effect	ns	**
Interaction	ns	ns

* : There is a tendency of difference between means ($p < .05$)

** : There is a significant difference between means ($p < .01$)

ns : No significant difference between means

Table II Median of plasma nitrate concentration for each intensity of parasitaemia score for *T.congolense* infected sheep

Buffy Coat (Score)	Nitrate concentration (μM)
0	17.5
1	15.0
2	16.0
3	15.1
4	16.4
5	26.5

Kruskal Wallis: $H = 5.63$, d.f. = 5, $p = 0.345$

Figure 1: Nitrate (Γ^{-1}) of *T.congolense* infected sheep fed diet a normal (NI) or a urea supplemented (UI) diet and their respective controls NC and UC

