University of Glasgow Veterinary School

Report

The effect of dietary arginine restriction on parasitaemia levels in mice infected with *Trypanosoma congolense*

by Geert Wassink (UGVS)

Appendix III

The effect of dietary arginine restriction on parasitaemia levels in mice infected with *Trypanosoma congolense*

Report **27 February, 1996**

Geert Jan Wassink **Department of Veterinary Physiology**
University of Glasgow Veterinary School

Background

The highly inducible enzyme ornithine decarboxilase catalyses the conversion of ornithine to the polyamine putrescine which is the initial and normally rate-limiting step in polyamine biosynthesis. Polyamine synthesis is required for normal cell growth and division. Drugs which inhibit the enzyme ornithine decarboxylase like efluornitine (α-difluoroomethhylornitine, DFMO, Ornidyl$^{(R)}$) have been shown to inhibit the growth of African trypanosomes (Bacchi et al., 1980).

Only small amounts of ornithine is provided by the diet and most is synthesised from arginine via the enzyme arginase and from glutamate via ornithine amino transferase through a glutamyl-γ-semialdehyde intermediate in the intestinal mucosa (Jones, 1985).

A significant amount of arginine is provided by the diet and adult mice placed upon an arginine-free diet have been shown to have markedly reduced levels of arginine and ornithine in a variety of tissues (Monso and Rubio, 1989). In recent research it has been shown that mice restricted in dietary arginine showed diminished tumorigenesis as a result of lowered pools of ornithine and the subsequent inhibition of putrescine biosynthesis (Gonzalez and Byus, 1991).

Restricted amounts of dietary arginine may also have an effect on the nitric oxide synthesis of macrophages since this is based on the conversion of arginine to nitric oxide and citrulline. Vincendeau and Daulouede (1991) reported that the macrophage cytostatic effect on *Trypanosoma musculi* involved a L-arginine dependent mechanism. The same authors (Vincendeau *et al.*, 1992) also reported that the in vitro cytostatic effect on *Trypanosoma brucei gambiense* and *Trypanosoma brucei brucei* depended on nitric oxide. On the other hand, inhibition of nitric oxide synthesis has been found to lead to reduced parasitaemia in mice infected with *T.brucei* (Sternberg et al., 1994).

In this experiment the hypothesis was investigated that *T.congolense* infected mice fed an arginine-deficient diet would show lower numbers of parasites than infected mice fed a normal diet because arginine is considered to be a requirement for parasite growth. To see whether this was due to an inhibition of putrescine synthesis or other factors an extra group of mice on a restricted arginine diet received putrescine in the water.

Materials and methods

Special diets

The specific amino acids in the diets by percentage of weight were:

Control Diet		**Arginine Deficient Diet**
cornflour snowflake	57.6	57.6
corn oil	8.0	8.0
dextrin	5.0	5.0
sulkafloc	5.0	5.0
sugar gran	5.0	5.0
AIN 76 vit mix	1.0	1.0
AIN 76 min mix	4.0	4.0
L-arginine	1.2	0.0
L-glutamic acid	1.2	2.4
glycine	1.2	1.2
L-histidine	1.2	1.2
L-isoleucine	1.2	1.2
L-leucine	1.2	1.2
L-lysine	1.2	1.2
methionine	1.2	1.2
L-phenylalanine	1.2	1.2
L-threonine	1.2	1.2
L-tryptophan	1.2	1.2
L-valine	1.2	1.2

Three weeks before the trypanosome infection the animals were put on the diet. The diets were in the powdered form and were offered *ad libitum* to the mice. The mice were weighed on Mondays and Thursdays.

Animals

Twenty five male, adult mice of approximately 40 grams were divided into five groups of 5 mice. The mice were housed individually.

Group 1: arginine-free diet + *T.congolense* infection

Group 2: arginine free diet + 1% putrescine H_2O + *T.congolense* infection

Group 3: complete amino acid control diet + *T.congolense* infection

Group 4: arginine-free diet

Group 5: normal diet

Infection

Mice in the groups 1, 2 and 3 were infected with *T.congolense* 1180 (GRVPS 57/17) isolated in Serengeti, Tanzania (Nantulya *et al.*, 1984). Each mouse was inoculated with 1 x 10^4 trypanosomes in 0.2 ml phosphate buffered saline (PBS) (containing 1.5% glucose). The infection lasted 17 days after which the animals were humanely killed.

Parasitaemia

Parasitaemias were determined three times a week using the improved Neubauer haemacytometer. One microliter (µl) of mouse blood, taken from the tail, was diluted 100 times in PBS containing 1.5% glucose.

Results

One of the animals in group 3 was not used in the final analysis because of a problem with its tail.

Body weight

The mice on the synthetic diets initially lost some body weight (figure 1) which resulted in the weights being slightly lower during the pre-infection period compared to the animals on the normal control diet. The decrease in body weight was highest in the animals fed the arginine free diet plus 1% putrescine (group 2) and the body weight of that group was significantly lower pre-infection than in the group receiving the normal control diet (group 5; $p<0.05$). After infection the body weights of the mice on the synthetic diets had stabilised but were significantly lower than the ones on the normal control diet (group5; $p<0.05$).

Parasitaemia

Parasites were detected in 4 out of 5 animals in the group receiving the synthetic arginine free diet on day 7 after infection, whereas the animals in the other two groups were all negative (figure 2). After 17 days one of the animals in the group receiving arginine free diet plus 1% putrescine in the water and two animals in synthetic control diet were still negative. Average parasitaemia was significantly higher in the group receiving the synthetic arginine free diet than in the group on the synthetic control diet ($p<0.05$). The parasitaemia of the group fed the arginine free diet plus putrescine in the water appeared to be intermediate (table 1).

Discussion

The results show the opposite of what was hypothesised at the start of the experiment. Instead of the expected restricted cell growth of the parasites due to the limited availability of arginine to the mice, the parasites were found earlier and more parasites were counted than in the mice fed the complete synthetic control diet. In two of the four control mice the parasites

had not established themselves on day 17 whereas the infected mice on the arginine free diet were all positive. The parasitaemia of the group on the arginine free diet plus 1% putrescine in the water should be read with caution since the animals lost more weight pre-infection compared with the other groups. Infection did not result in a further decrease in body weight in all three infected groups possibly due an increase in spleen size.

These findings are different from the findings in research into murine epidermal carcinogenesis in which a reduced number of tumours were found after feeding a diet restricted in arginine (Gonzalez and Byus, 1991). It is not clear from the present experiment whether the arginine free diet diminished nitric oxide production in the mice but if this were the case it did not significantly reduce parasitaemia. Sternberg *et al.* (1994) reported that inhibition of nitric oxide synthesis leads to reduced parasitaemia in *T.brucei* infected mice, possibly through the inhibition of the immuno-suppressive effect of nitric oxide. Under in vitro conditions several researchers (Mabbott *et al.*, 1994; Vincendeau *et al.*, 1991, 1992; Sternberg *et al.*, 1994) have found a cytostatic effect of nitric oxide on trypanosomes, however, Mabbott et al. (1994) and Sternberg *et al.* (1994) found this is not the case under in vivo conditions. It appears that arginine, apart from being a precursor for putrescine, is necessary for the resistance of the mice to the trypanosome infection. Further experiments are necessary to see whether these results are repeatable.

Literature

Bacchi, C.J., Nathan, H.C., Hutner, S.H., McCann, P.P., Sjoertsma, A. (1980):
Polyamine metabolism: A potential therapeutic target in trypanosomes.
Science 210: 332-334.

Gonzalez, G.G. and Byus, C.V. (1991):
Effect of dietary arginine restriction upon ornithine and polyamine metabolism during two-stage epidermal carcinogenesis in the mouse.
Cancer Research 51, 2932-2939.

Jones, M.E. (1985):
Conversion of glutamate to ornithine and pyrroline-5-carboxilate, a possible modulator of arginine requirements.
Journal of Nutrition 115, 509-515.

Mabbott, N.A., Sutherland, I.A., Sternberg, J.M. (1994):
Trypanosoma brucei is protected from the cytostatic effects of nitric oxide under in vivo conditions.
Parasitology Research 80, 687-690.

Monso, E. and Rubio, V. (1989):
Participation of ornithine aminotransferase in the synthesis and catabolism of ornithine in mice.
Biochemistry Journal 259, 131-138.

Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R. and Moloo, S.K. (1984):

Resistance of cattle to tsetse-transmitted challenge with *Trypanosoma brucei* or *Trypanosoma congolense* after spontaneous recovery from seringe-passaged infections.

Infection and Immunology 43, 735-738.

Sternberg, J., Mabbott, N., Sutherland, I. and Liew, F.Y. (1994):

Inhibition of nitric oxide synthesis leads to reduced parasitaemia in murine *Trypanosoma brucei* infection.

Infection and Immunity, May 1994, 2135-2137.

Vincendeau, P. and Daulouede, S. (1991):

Macrophage cytostatic effect on *Trypanosoma musculi* involves an L-arginine-dependent mechanism.

Journal of Immunology 146, 4338-4343.

Vincendeau, P., Daulouede, S., Veyret, B., Darde, M.L., Bouteille, B. and Lemesre, J.L. (1992):

Nitric oxide-mediated cytostatic activity on *Trypanosoma brucei gambiense* and *T.brucei brucei*.

Experimental Parasitology 75, 353-360.

Table 1: ^{10} log parasitaemia ± standard deviation of *T.congolense* infected mice fed special synthetic amino acid diets and their controls

Group	^{10} log Parasitaemia
Synthetic arginine free diet + *T.congolense* Infection	6.7 ± 1.5^{a}
Synthetic arginine free diet + *T.congolense* Infection + 1% Putrescine H_2O	4.0 ± 2.4^{b}
Synthetic control diet + *T.congolense* Infection	1.8 ± 2.6^{a}

Means of groups with the same letter are significantly different $p<0.05$

Figure 1: Average body weight (grams) of *T.congolense* infected mice fed a synthetic arginine free diet (group 1), a synthetic arginine free diet plus 1% putrescine in the water (group 2), a synthetic control diet (group 3) and uninfected mice fed a synthetic arginine free diet (group 4) and a normal control diet (group 5)

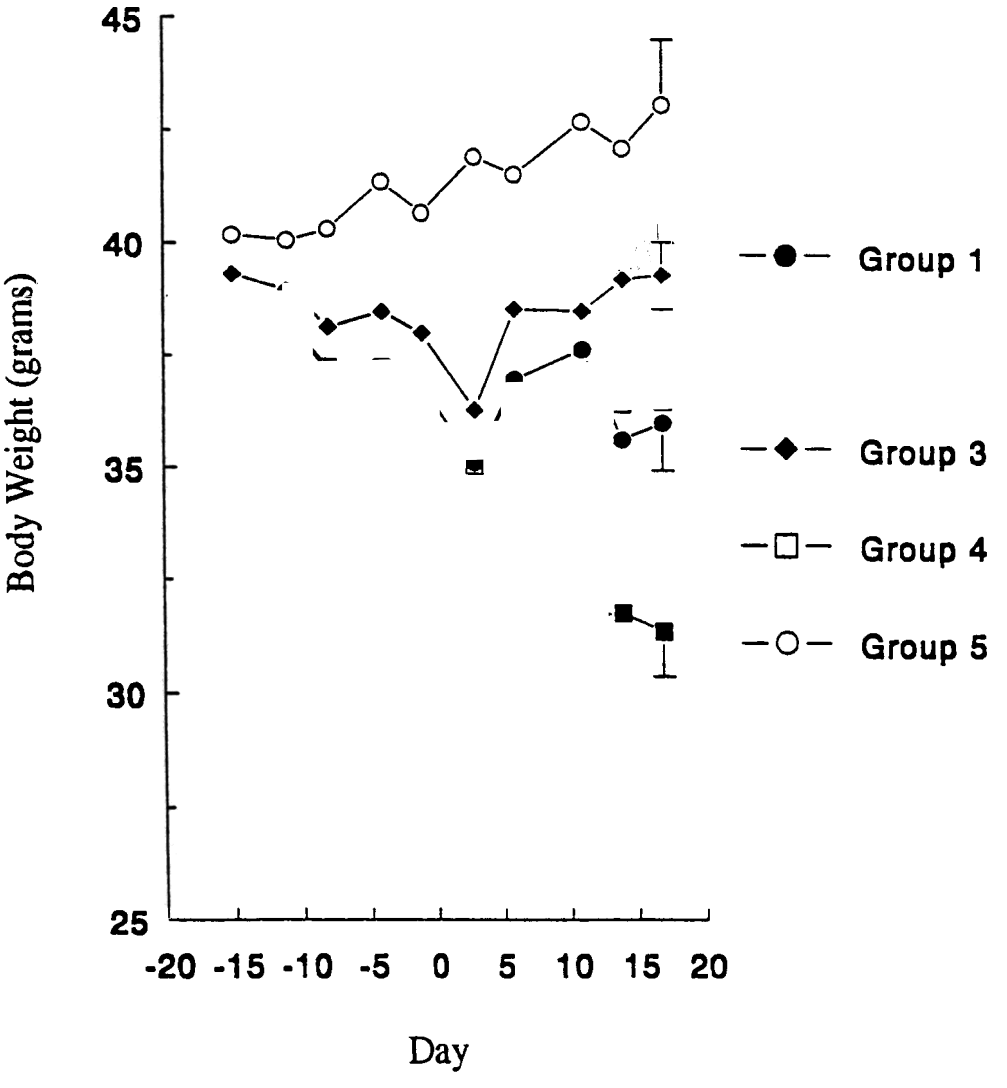

Figure 2: Average ^{10}log parasitaemia of *T.congolense* infected mice fed a synthetic arginine free diet (group 1), a synthetic arginine free diet plus 1% putrescine in the water (group 2) and a synthetic control diet (group 3)