Effects of Medication with Ivermectin, Chloroquine and Artemether on Plasma Nitrate / nitrite Levels in Calves Naturally Infected with *Onchocerca Gutturosa*

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Abstract

In a comparative study involving the use of Ivermectin, Chloroquine and Artemether against Onchocerca gutturosa in calves, the plasma nitrate /nitrite concentration was measured. Following treatment and clearance of skin mf of O. gutturosa, the plasma nitrate/ nitrite concentrations, nor the stable end product of Nitric Oxide (NO) breakdown, rise significantly although it showed short peaks following reduction in dermal mf counts but no clear correlation was detected.

Introduction

The production of nitric oxide (NO) by activated macrophages has been identified as a factor mediating proliferation suppression in parasitic infection (Pfaff et al., 2000). Nitric oxide regulates the development of immune responses either directly by inducing subsets of T- cells or Ag-presenting cells or indirectly through the modulation of cytokine secretion (Pfaff et al., 2000). It has been demonstrated that increased production of IFN-γ leads to high production of NO which in turn inhibits the production of IL-12 by activated macrophages with a negative feedback mechanism which may operate in mf-infected animals (Richard et al., 2000). Piedrafita et al. (2004) reviewed the role of antibody-dependent cell cytotoxicity, in rats against Fasciola hepatica, which mediated by NO produced by activated monocytes and macrophages and they concluded that fasciola species modulate the host immune response and down-regulate type1 responses during infection. Moreover, McGarry et al. (2005) showed, for the first time, that inducible NO is essential for the rapid sequestration of mf by Di-ethylcarbamazine (DEC).

In this study we measured the plasma nitrate /nitrite concentrations, the end product of nitric oxide, and its association with the mf clearance was discussed.

Materials and methods

1. Experimental animals:

A total of 15 male Zebu calves, 2-3 years old, naturally infected with Onchocerca gutturosa were used. The animals were divided into three equal groups each of five calves; they were kept at the premises of CVRL, Khartoum, and had free access to water and sorghum straw. Blood samples and skin snips were collected before treatment (day 0) then they were weighed and treated according to the following regimen: Group I received I/M Chloroquine (Chloroquine – Phosphate Base - France Lab) at a dose rate of 200 mg/day for 7 consecutive days and then weekly for 6 weeks. Group II received weekly S/C Ivermectin (Ivomec®, Merck Sharp & Dohme, New Jersy, USA) at a dose rate of 150 µg/kg body weight for 6 weeks; whereas Group III, received daily I/M Artemether (Artemedine, Kunming Pharmaceutical Corp- China) at the dose rate of 160 mg/animal/day for three successive days. After each treatment the animals were monitored for 2 hours for any drug- induced reactions.

2. Blood samples:

Calves were bled at the jugular vein and 5 ml whole blood were withdrawn in vacutainers with EDTA at 1, 2, 4, 6, 24, 48, and 72 hours post-treatment then weekly for six weeks. Each blood sample was immediately centrifuged at 3000 rpm for 15 minutes and plasma samples were stored at –20 oC for analysis.

3. Measurements of plasma nitrate /nitrite:

Plasma nitrate/nitrite concentration was measured using the method of Diven, et al.(1962) and Farih (1973) .

a. Reagents:

Mercuric chloride solution 5% Hgcl containing 1.5 ppm of copper as cupric sulfate; powder Zinc; Sulfanilamide in 20% HcL; color reagent-0.02% solution of N- (1- naphthal)- ethylenediamine dihydrochloride .

b. Method:

Protein free plasma was prepared by adding 0.5 ml mercuric chloride to 0.5 ml plasma (or serum) and 4 ml distilled water. For nitrate determination the protein free plasma or serum prepared by the same method except that a pinch of powdered zinc was added after the addition of mercuric chloride, the mixture was mixed until it became gray in color, suggesting that the oxidation-reduction was completed within 7- 10 minutes. The precipitated protein was removed by centrifugation at 3000 rpm for 5 minutes. Sulfanilamide solution (0.5 ml) was added to the protein-free plasma solution, well mixed, then 0.5 ml of color reagent was added and the volume was completed with distilled water to 5 ml. Maximum color development was obtained within 10 minutes and stable for 2 hours. The amount of red color was measured as optical density (O.D) at 520 nm using UV spectrophotometer (Jenway, 6105 / U.K.).

A standard regression curve was prepared by using known serial concentrations of sodium nitrate, against the O.D concentrations. The values of nitrite / nitrate were then calculated.

Results and Discussion

The mean plasma nitrate/ nitrite concentrations of calves before medication were 0.139, 0.255 & 0.233 µg/ml for Chloroquine, Ivermectin and Artemether treated group, respectively. After treatment an irregular increase and decrease in nitrate /nitrite concentration was observed. In Chloroquine -treated calves the nitrate concentration remained essentially above the pretreatment level and peaked at 24 hours post treatment (Figure 1) coinciding with the peak count of skin mf of O. gutturosa (359% of its initial count) and again a short peak was noticed at week 2 post treatment (Figure 1). In this group the mean concentration of nitrate / nitrite found to range between 0.095 and 0.311 µg/ml with a mean value of 0.196±0.055µg/ml.

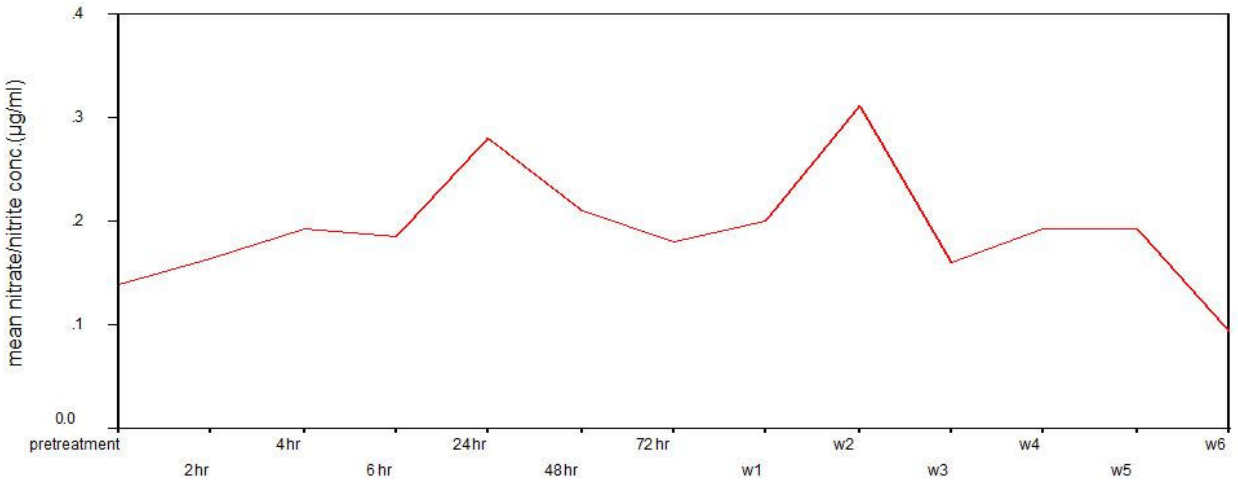

Figure 1 Nitrate /nitrite concentration in Chloroquine- treated group

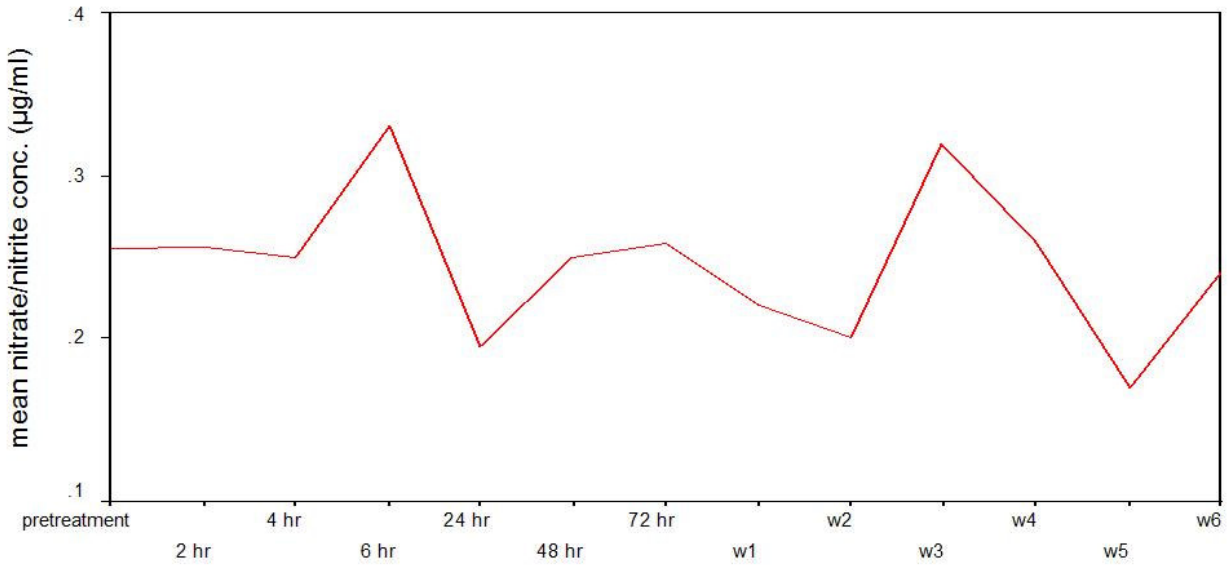

Figure 2: Nitrate /nitrite concentration in Ivermectin- treated group

In case of Ivermectin-treated calves the skin mf count showed rapid clearance within the first two days post treatment (15% of its initial count). The concentration of plasma nitrate/nitrite remained within the pretreatment level except for short peaks at 6 hours and week 3 post treatment (Figure 2) and it ranged between 0.170 to 0.330 µg/ml throughout the sampling period with a mean concentration of 0.256± 0.046 µg/ml. In Artemether-treated group two sharp peaks of nitrate/nitrite occurred at 48 hours and week 1 post treatment (Figure 3). and ranged between 0.140 to 0.521 with a mean concentration of 0.258± 0.113 µg/ml. The obtained results may show a relationship between nitric oxide derivatives and mf clearance in treated calves. However, Winkler et al. (1998) reported consistent increase in serum nitrate/ nitrite concentrations in human filariasis treated with Ivermectin. In another study Taylor et al. (1996) showed that Brugia malayi and Onchocerca lienalis were highly susceptible to nitric oxide in vitro. However, the role of nitric oxide in regulating the development of immune responses was discussed in previous investigations (Pfaff et al., 2000; MC Garry et al. 2005) and its action was

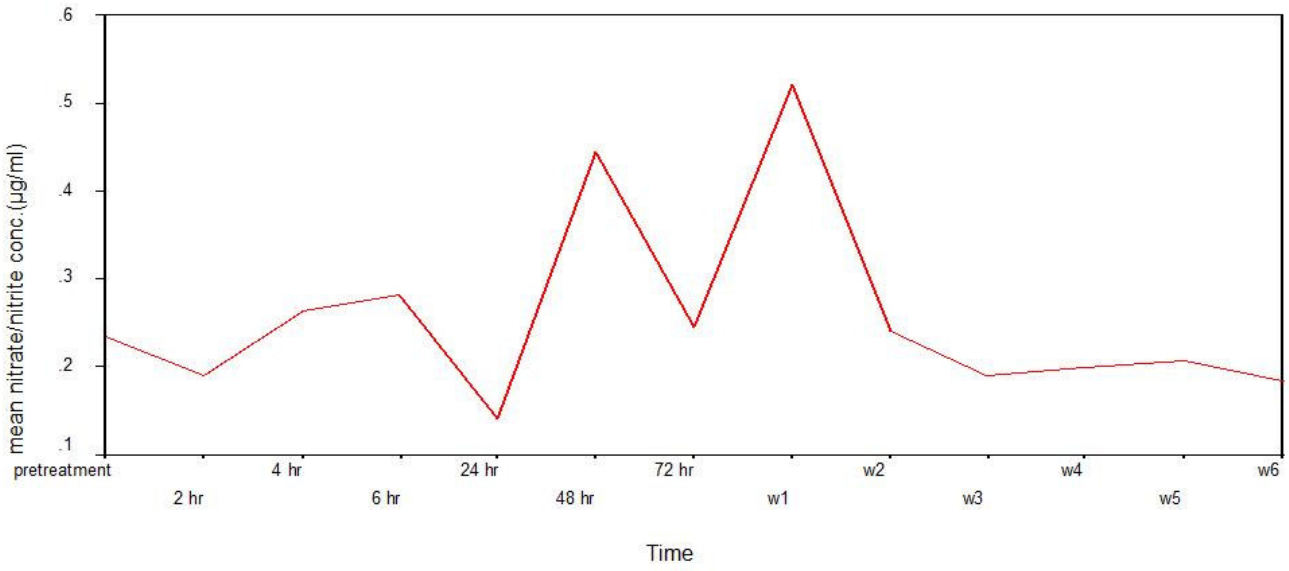

Figure 3: Nitrate /nitrite concentration in Artemether- treated group

shown to be either directly by inducing T cells and Ag -presenting cells (APC) or indirectly through the modulation of cytokines secretion. Such regulation was shown to have a negative feedback mechanism governed by the amount of released antigens (Pfaff et al., 2000). In this study although the concentration of nitrate/ nitrite is inconsistent, yet it could be of value to study the possible role of nitric oxide in the mechanism of mf death. More studies are suggested in this issue.

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