Haemodynamics of hamsters during *Ancylostoma ceylanicum* infection

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Abstract. Alterations in haematological and liver glycogen values during the course of *Ancylostoma ceylanicum* (100L, p.o.) infection in hamsters were investigated. In early phase of infection, there were marginal changes in haematological parameters, albumin-globulin ratio and liver glycogen, which transformed into severe alterations on patency (day 18, post infection). Haemoglobin, packed cell volume, total erythrocyte counts and liver glycogen values had a decreasing trend and reticulocytes increased considerably to compensate the red blood cell loss. Albumin/globulin ratio which was in favour of albumin in the beginning, reversed on day 24 p.i. When the animals were deparasitized with mebendazole (5 mg/kg x1, p.o.), all the parameters started resuming normalcy and on day 12 of treatment of all the parameters except albumin/globulin ratio attained the pre-infection level.

Keywords. *Ancylostoma ceylanicum*; hamster; haemodynamics; liver glycogen.

1. Introduction

Infection with gastrointestinal helminths inflict a variety of structural and functional changes in the intestine (Symons 1969) and blood of hosts (Ogilvie et al 1978; Roth and Levy 1980). Qualitative and quantitative changes in haematological picture constitute some of the most frequent and easily observed manifestations of infection and also reflect the immune response of the host to infection (Boyer et al 1971; Ogilvie et al 1978; Moqbel 1980). Since, *Ancylostoma ceylanicum* a hookworm of dog, cat and man is of relatively recent introduction to the experimental field (Ray et al 1972; Visen et al 1984; Gupta et al 1987; Srivastava et al 1986) and gained favour for experimentation over other hookworm models, *Nippostrongylus brasiliensis* in rats and *Nematospiroides dubius* in mice (Ray et al 1972; Misra et al 1981; Katiyar et al 1982, 1984) there was need to understand haematological and related parameters of the infected host (hamster). A detailed study on this aspect is also important because this laboratory host is the most suitable for *A. ceylanicum*.

Accordingly an attempt has been made to monitor alterations in haematological parameters and liver glycogen reserve of hamsters during the course of infection and following anthelmintic therapy.

2. Materials and methods

2.1 Animals

Laboratory bred male hamsters (70–85 g) maintained under standard animal house conditions, served as experimental host.
2.2 *Ancylostoma ceylanicum*

The procurement of infective larvae, infection to host and recovery of worms have already been dealt in detail elsewhere (Gupta et al 1987; Srivastava et al 1986).

2.3 **Experimental design**

Two groups of animals were examined. Group I (infected) comprising of 60 hamsters, was infected orally with $100\pm 5L_3$. Three of the survivors from this group were exsanguinated at 3 days interval from day 3–30 p.i. (10 intervals) and their blood samples were collected for examining haematological parameters [Haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), reticulocyte percentage (RP), differential leucocyte count (DLC) and albumin/globulin ratio (A/G)]. Liver was isolated under chilled condition for estimating glycogen.

On day 18, when the infection attained patency (Visen et al 1984; Gupta et al 1987) in the intestine, the remaining animals of Group I (infected) were divided into two sub groups. The animals of one sub group were deparasitized with mebendazole (5 mg/kg/d, per os) and those of the other sub group received sham treatment of normal saline and examination continued till 30 p.i.

Group II (uninfected) having identical number of animals and receiving mebendazole and sham treatment of D.W. was run parallel and examined simultaneously.

On autopsy after collecting blood and liver; intestine of each animal was also examined for observing the worm load. Two to three replicates were carried out.

2.4 **Haematological examination**

Hb was determined by Sahli-Hellige Haemoglobinometer. Total erythrocyte and leucocyte counts were made on heparinized blood using standard dilution methods (Hayem’s fluid for RBC and Turk’s fluid for WBC) on a Neubauer haemocytometer (Anon 1978). The differential leucocyte count was made on films of non heparinized blood, fixed in methanol and stained with 10% Giemsa stain. Packed cell volume was determined with heparinized blood in Wintrobe tubes (Anon 1978). The reticulocytes were counted as described by Cheema and Scofield (1984).

2.5 **Determination of A/G ratio**

The test sera were first electrophoresed on cellulose acetate membrane in microzone electrophoretic apparatus (Beckman) (Briero and Mull 1964; Jane 1979) and later were scanned over the scanning densitometer (R-112) at 520 nm.

2.6 **Estimation of liver glycogen**

Liver glycogen (LG) was extracted from 25–50 mg isolated liver (Good et al 1933) and estimated colorimetrically (Montgomery 1957).
3. Results

The sequential changes in various parameters that occurred during the course of infection have been depicted in table 1.

3.1 Hb

Hb level remained unaltered up to day 6 of infection. On day 9 slight decrease was seen which became pronounced as the infection reached patency i.e. on day 18. Maximum drop was observed on day 24 of infection (infected group - 4.50 ± 0.94 g%, control group - 17.59 ± 0.98 g%).

Mebendazole, knocked out all the worms, and Hb started rising from day 3 post-treatment and reached pre-infection level on day 12 post-treatment.

3.2 TEC

TEC showed a gradual fall which was significant on day 12 (when L_5 stage was present). On day 18 when adult worms started sucking blood, the fall was more conspicuous and maintained a plateau up to day 30 (last observed period).

3.3 TLC

Very mild alteration was recorded and the maximum rise (46.5% over the control) was observed on day 12 of infection.

3.4 DLC

The differential count remained more or less unaltered during the course of infection.

3.5 PCV

Decrease in PCV started from day 9 and continued till the day of last observation (day 30 p.i.) where the value of infected group was 8.25 ± 1.09% as compared to 48.39 ± 5.31% in control group.

3.6 RP

The reticulocytes were very few in uninfected hamsters (mean value - 1 ± 0.68%) but in the infected animals, a dramatic rise was recorded from day 15 of infection and attained peak (i.e. 78 ± 3.74%) on day 30 p.i.

3.7 A/G ratio

The A/G ratio which in the initial phase was in favour of albumin (mean value 1.47 ± 0.52) levelled off on day 15 p.i. (mean value 1.05 ± 0.19) and subsequently turned in favour of globulin on day 24 (0.88 ± 0.14).
Table 1. Dynamics of blood parameters and liver glycogen reserve of male hamsters during *A. ceylanicum* (100 L2/p.o) infection.

<table>
<thead>
<tr>
<th>Parameters observed (pooled control values)</th>
<th>Observation intervals (days)</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><strong>Hb g%</strong> (17.59 ± 0.98) Infected treated</td>
<td>16.73 ± 0.81</td>
<td>16.70 ± 1.31</td>
<td>14.63 ± 1.17</td>
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<tr>
<td></td>
<td>8.88 ± 2.05</td>
<td>10.3 ± 2.69</td>
<td>15.1 ± 2.80</td>
</tr>
<tr>
<td><strong>TEC (× 10⁶)</strong> (6.13 ± 0.42) Infected treated</td>
<td>5.40 ± 0.09</td>
<td>5.39 ± 0.10</td>
<td>5.64 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>3.45 ± 0.53</td>
<td>3.39 ± 0.26</td>
<td>4.8 ± 1.24</td>
</tr>
<tr>
<td><strong>TLC</strong> (5189 ± 1475) Infected treated</td>
<td>5637 ± 543</td>
<td>5859 ± 421</td>
<td>5791 ± 264</td>
</tr>
<tr>
<td></td>
<td>5808 ± 896</td>
<td>5587 ± 589</td>
<td>5800 ± 699</td>
</tr>
<tr>
<td><strong>PCV %</strong> (49.39 ± 5.31) Infected treated</td>
<td>48.83 ± 0.95</td>
<td>49.0 ± 2.15</td>
<td>39.66 ± 4.41</td>
</tr>
<tr>
<td></td>
<td>27.33 ± 3.25</td>
<td>35.5 ± 2.29</td>
<td>40.5 ± 6.73</td>
</tr>
<tr>
<td><strong>Reticulocyte %</strong> (100.0 ± 0.68) Infected treated</td>
<td>0.90 ± 0.2</td>
<td>0.67 ± 0.32</td>
<td>1.66 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>28.66 ± 16.83</td>
<td>5.65 ± 1.75</td>
<td>2.63 ± 1.04</td>
</tr>
<tr>
<td><strong>A/G</strong> (1.50 ± 0.35) Infected treated</td>
<td>1.47 ± 0.52</td>
<td>1.38 ± 0.65</td>
<td>1.35 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>1.07 ± 0.11</td>
<td>1.10 ± 0.19</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td><strong>Liver glycogen mg/g</strong> (74.73 ± 8.76) Infected treated</td>
<td>68.86 ± 4.37</td>
<td>66.27 ± 13.5</td>
<td>39.39 ± 7.61</td>
</tr>
<tr>
<td></td>
<td>16.77 ± 4.9</td>
<td>51.87 ± 4.5</td>
<td>58.41 ± 18.04</td>
</tr>
</tbody>
</table>

Mean value ± SE.
Haemodynamics of hamsters during A. ceylanicum infection

From day 6 of infection, there was gradual depletion of glycogen reserve from the liver and on day 9, almost half of the reserve was exhausted ($39.39 \pm 7.61 \text{ mg/g}$ vs $74.73 \pm 8.76 \text{ mg/g}$). The depletion was proportionate to the age of infection and almost all the glycogen was utilized by day 27 p.i. ($1.84 \pm 1.38 \text{ mg/g}$).

4. Discussion

Haematological changes represent a facet of the host's overall response to infection. In gastrointestinal helminthiasis this aspect has been well documented, firstly from a description point of view (Ruitenberg et al. 1977; Moqbel 1980; Ogilvie et al. 1980; Przyjalkowski 1980; Cheema and Scofield 1984) and secondly in an attempt to pinpoint the cells that may be important for host defence (Ogilvie et al. 1980; Wakelin and Donachie 1983).

The changes that occurred in the TEC, PCV and Hb parameters suggested the development of microcytic anaemia which is characteristic of blood sucking hookworms (Chatterjee 1967; Roach 1970; Hoagland and Schad 1978). These parameters showed the abducting trend after day 12 of infection when cutting armature of parasite was fully developed and they started removing the blood.

The marked increase in reticulocytes during patency may be due to heavy destruction of erythrocytes, which consequently forced the reticulocytes to accumulate in the blood to compensate erythrocytic loss.

The elevated leucocytes in early phase of infection was in conformation with the previous observations of Baker (1962), Ogilvie et al. (1978) and Roth and Levy (1980) but dipping at the later stage of infection could be due to the severe loss of cellular components of the blood because of haemophagy by parasite.

The reversion of A/G ratio at the later stage of infection depicted the development of protective immunity (Gupta and Katiyar 1985). Carroll and Grove (1985) also observed increased immunoglobulins in dogs following A. ceylanicum infection. Non resuming of A/G ratio after mebendazole therapy may be explained on the basis of definite half life of antibodies. Therefore, as soon as the antigenic source is removed, the globulin levels are not supposed to reach preinfection level.

Excess of carbohydrate is stored in the liver as glycogen and is released during emergencies. The excessive blood loss during A. ceylanicum infection and consequent drainage of glucose along with, necessitated an additional demand of glycose by the host. Hence, the liver glycogen was mobilized to meet the host requirement (Kaul et al. 1982). As such, the reserve glycogen in the liver was depleted and showed decreasing pattern.

It may be inferred that A. ceylanicum like other hookworms of men and animals, is highly pathogenic to its laboratory host, hamster, suggesting the suitability of this host parasite combination for experimental studies.

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