Effects of a protein-free diet on worm recovery, growth, and distribution of *Echinostoma caproni* in ICR mice

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Abstract

The effects of a protein-free diet on the host–parasite relationship of *Echinostoma caproni* in ICR mice were studied. The experimental diet was a customized protein-free diet (PFD) in pellet form containing 0% protein. The control diet consisted of a standard laboratory diet containing 23% casein as a source of protein. A total of 24 mice were each infected with 15 metacercarial cysts of *E. caproni*. Twelve mice were placed on the experimental diet (experimentals) and the remaining mice (controls) were placed on the control diet. Experimental and control mice were necropsied at 2, 3, and 4 weeks postinfection (p.i.). The weight of mice on the PFD was markedly lower than that of mice on the control diet. The length and circumference of the small intestine of infected mice on the PFD were significantly lower than those of the controls at 3 weeks p.i. (Student's t-test; $P < 0.05$). Worm recoveries from mice on the PFD were significantly lower than those of the controls at 3 weeks p.i. There was a significant decline in worm body area in worms from the mice on the PFD compared with those on the control diet at 2, 3, and 4 weeks p.i. Worm dry weights from mice on the PFD were significantly lower than those on the control diet at 2 weeks p.i. Worms from hosts on the PFD were located more posteriad in the gut than those recovered from mice on the control diet. The findings suggest that the PFD contributes to growth retardation of *E. caproni* in ICR mice.

Introduction

Sudati *et al*. (1996, 1997) reviewed the salient literature on the effects of host diet on helminth growth and development and noted that most work on this topic was done on cestodes and nematodes in various vertebrate hosts. Relatively little information is available on gastrointestinal trematodes in rodent hosts.

Echinostoma caproni is a useful organism for studies on growth and development of a gastrointestinal trematode in a murine host (see Fried & Huffman, 1996 for review). Sudati *et al*. (1996, 1997) used this model to study the effects of high lipid and high protein diets, respectively in ICR mice.

We are currently concerned with the effects of low or zero protein diets on the growth of *E. caproni* in ICR mice. The literature shows that studies on the effects of low protein diets on helminths grown in vertebrate hosts are confined to various species of gastrointestinal nematodes and two schistosome species (Dobson & Bawden, 1974; Slater, 1988; Michael & Bundy, 1991, 1992 a,b; Gerges *et al*., 1994; Coutinho *et al*., 1997; Johansen *et al*., 1997). The results of these studies are disparate and depend on the species of helminth and vertebrate used; generalizations of the findings of these studies are not possible.

Graczyk & Fried (1998) examined the salient literature on human echinostomiasis and concluded that it is a

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common but forgotten food-borne disease. Because echinostomiasis may occur in people from socioeconomic groups that suffer from protein malnutrition, studies on the effects of low or zero protein diets on *E. caproni* seemed appropriate. The purpose of this study was to examine the effects of a protein free diet on worm recovery, growth, and distribution of *E. caproni* in ICR mice.

Materials and methods

Metacercarial cysts of *Echinostoma caproni* were removed from the kidney/pericardial region of experimentally infected *Biomphalaria glabrata* snails and fed by stomach tube (15 cysts per mouse) to 6–8-week-old female ICR mice (Manger & Fried, 1993). The experimental mice were fed a customized protein-free diet (PFD) in pellet form containing 54% cornstarch, 18% DYETROSE, 10% sucrose, 5% cellulose, 7% soybean oil, 3% salt, 2% choline bitartrate and 1% vitamins (Dyets Inc., Bethlehem, Pennsylvania). The control mice were fed a standardized rat–mouse–hamster 3000 diet (RMH) in pellet form containing 61% carbohydrate, 23% protein, 5% lipid, 5% salt, 4% fibre and 2% vitamins (US Biochemicals Co., Cleveland, Ohio). The total calorific value of each diet was about equal.

A total of 24 mice were used in the experiment; 12 mice were maintained on the PFD and the remainder on the control diet (RMH). On the day of infection, the mice were weighed and maintained four per cage on either the PFD or the RMH diet. Food and water were provided ad libitum. Four mice on the PFD and four mice on the RMH diet were each necropsied at 2, 3, and 4 weeks post infection (p.i.). Mice were weighed at necropsy 2, 3, and 4 weeks p.i. and the small intestine was removed from the pyloric sphincter to the ileocaecal valve and divided into five equal sections numbered 1–5, beginning with the pylorus. Length and circumference measurements of the small intestine of mice (n = 4 for each measurement) on both the PFD and RMH diets were made at 3 weeks p.i. Worms were removed from the intestine and the location and number of worms in each section were recorded at 2, 3, and 4 weeks p.i. Ten worms were selected at random from mice on the PFD and RMH diets at 2, 3, and 4 weeks p.i; they were rinsed in Locke's solution, fixed in hot (85°C) alcohol–formalin–acetic acid, stained in Gower's carmine, dehydrated in ethanol, cleared in xylene, and mounted in Permount (Kaufman & Fried, 1994). Length and maximum width measurements of worms were made with the aid of a calibrated ocular micrometer to give body area in mm^2 for both experimental and control groups at 2, 3, and 4 weeks p.i. (Sudati *et al*., 1997).

About 100 eggs were dissected from the uteri of worms grown for 2 weeks on the PFD and placed in artificial spring water (ASW) at 28°C for 10 days to determine if miracidia would develop and hatch (Idris & Fried, 1996).

Dry weights were determined on five randomly selected worms from mice on the PFD and RMH diets at 2 and 3 weeks p.i. Worms were dried at 110°C for 24 h and weights were determined based on four separate samples per group so that the mean ± SE could be determined. Whenever possible, differences in means were compared between groups using Student's t-test with $P < 0.05$ being considered significant.

Results

There was a marked difference in mice weights between the PFD and control groups at 2, 3, and 4 weeks p.i. The weights of the mice on the control diet increased steadily until 4 weeks p.i. whereas the weights of the mice on the PFD decreased steadily until 3 weeks p.i. Mice were weighed in groups of four so that only the average weights were determined. By 3 weeks p.i. mice on the control diet weighed about 1.5 times more than those on the PFD. All mice on both diets survived until necropsy. By 3 weeks p.i. mice on the PFD showed considerable hair loss in the posterior body region. The mean length of the small intestine (38.0 ± 0.7 cm) of mice on the control diet at 3 weeks p.i. was significantly greater than that of mice on the PFD (28.3 ± 1.2 cm). The mean circumference of the small intestine (1.3 ± 0.1 cm) of mice on the control diet at 3 weeks p.i. was significantly greater than that of mice on the PFD (0.9 ± 0.5 cm). At 3 weeks p.i., there was a significantly lower worm recovery in mice on the PFD than in the controls. Most worms from mice on the control diet were distributed in segments 3 and 4 at all weeks p.i. with none in segment 5. Most worms from mice on the PFD were in segment 4 at all weeks p.i. and some were recovered from segment 5. The mean body area of worms from hosts on the PFD were significantly smaller than those from the control mice at 2, 3, and 4 weeks p.i. The body areas of worms on both the PFD and control diets increased steadily with time. Worm weights from hosts on the PFD were significantly less than those of the controls at 2 weeks p.i.

All worms examined from the mice on the PFD at 2 weeks p.i. were ovigerous and contained 200–400 eggs each. Most eggs obtained from hosts on the PFD developed normally and produced miracidia as described in Idris & Fried (1996).

Discussion

Worms from hosts on the PFD, when compared with control worms, showed significant decreases in both body area and dry weight, findings similar to those of Sudati *et al*. (1997) on *E. caproni* in mice on high protein diets. In the present study, the guts of infected mice on the PFD were significantly smaller than those of the infected controls. Although histological studies were not done, the gross morphology of infected guts from hosts on PFD was more severely affected than that of the infected control guts. Along with the severe weight loss of mice on the PFD was extensive damage to the guts of hosts on PFD. These changes in gut characteristics probably contribute to a significant decline in worm growth compared to worms from hosts on the control diet. However, the fact that worms from hosts on the PFD showed reasonable growth and were fecund, attests to the fact that *E. caproni* can thrive under condtions of severe protein malnutrition in a murine host for up to 4 weeks. The implications in terms of human echinostomiasis is that under conditions of severe malnutrition the worms would probably survive reasonably well in man.

Mice maintained on the PFD showed hair loss, a condition previously described as 'wasting syndrome'

(Poleschchuk *et al*, 1988) in moustached tamarin monkeys (*Saguinus mystax*) infected with various helminths and maintained on a low protein diet (2 g protein per animal per day). The wasting syndrome was reversed by placing the animals on a high protein diet. The wasting syndrome seen in mice infected with *E. caproni* is probably characteristic of mice on the PFD.

Distribution data in this study, similar to what was seen in Sudati *et al*. (1997), suggested that there was a posteriad shift of worms with time in hosts on the PFD. This worm shift may contribute to worm rejection from these hosts since *E. caproni* is typically a parasite of the midgut rather than the hindgut region of mice.

Numerous studies on gastrointestinal nematodes in rodent and porcine hosts maintained on low protein diets (Slater, 1988; Michael & Bundy, 1991, 1992 a,b; Coutinho *et al*., 1997; Johansen *et al*., 1997) suggest that such diets interfere with the host's ability to produce antibodies and cellular defence products and generally favour the survival of the parasite in the host on the altered diet. Whether murine hosts infected with *E. caproni* and maintained on the PFD have such lowered immunological responses is not known at this time, but merits further work.

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