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Growth failure after recurrent fever in young guinea pigs

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Abstract

Infection causes fever and suppression of appetite, a combination of effects which threatens normal growth in infected children. We have used an animal model to study the effects on growth of recurrent simulated Gram-positive bacterial infection. After weaning, 10 guinea pig pups underwent surgery under general anaesthesia for the implantation of temperature-sensitive radiotelemeters and thereafter were assigned to receive intramuscular injections of either 50 μg/kg muramyl dipeptide (MDP), or sterile saline. During a 30-day period corresponding to their rapid growth phase, the pups were given eight injections. MDP resulted in fevers of about 1.5 °C on each occasion, but no significant change in body temperature occurred after saline injections. Food intake was suppressed during each febrile episode such that 24-h intake was significantly lower on days of injections of MDP, compared to days between MDP injections in the same animals, and compared to that of animals injected with saline. The rate of weight gain of the MDP-injected guinea pigs was significantly lower than that of the control group and failed even to achieve a rate similar to the saline-injected group in their more adult-like growth phase. Plasma zinc concentration was significantly lower in MDP- compared to saline-injected animals sampled 8 days after the last injection. Our results show that recurrent fever during the growth phase of young guinea pigs results in irreversible growth failure, and that reduced food intake on days when the animals were febrile was at least partly responsible for this reduced rate of growth.

Keywords: Growth failure; Fever; Body mass; Food intake; Guinea pig; Muramyl dipeptide; Zinc

1. Introduction

Infection in early childhood is associated with weight loss, and repeated infection with poor growth and malnutrition [1]. One might expect the greatest weight loss in children with infections of the gastro-intestinal tract, but many other non-diarrhoeal infections too, have a substantial impact on weight loss in children, contributing to growth failure [1–4].

Infections frequently are accompanied by fever and in children the degree of weight loss is positively correlated with the occurrence of fever [2]. Metabolic rate increases by about 13% per 1 °C rise in body temperature in humans [5] and in children would potentially divert energy from the needs for growth, unless food intake is sufficient to sustain both the rise in metabolic rate during fever [6], and the metabolic demands of growth. Such a scenario is unlikely, however, because of the "anorexia of infection" [3] now accepted to be part of the suite of sickness behaviours which accompany infection [7]. In one study, appetite was found to be suppressed by about 20% in young children during a bout of infectious illness [8].

Single bouts of infection may indeed cause short-term weight-loss, but recurrent bouts, common in the young of many developing communities, might be expected to have more serious and long-term consequences for growth. We have used an animal model to simulate recurrent febrile illness in the young. We induced fevers repeatedly in young guinea pigs, during a period corresponding to the animal's rapid growth phase [9] and measured food intake and body weight. We report here that animals which experienced repeated bouts of simulated infection, abrogated their juvenile growth spurt, at least partly because of decreased food intake on days of fever episodes, and lack of compensatory increased feeding between episodes.

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2. Materials and methods

2.1. Experimental animals and surgery

Ten young male and female guinea pigs (*Cavia porcellus* Dunken Hartly strain) were used in this study. Birth weights ranged between 34 and 100 g. Initially the pups were housed in cages with their mothers, in a room with a 12 h:12 h light: dark cycle with lights on at 07:00 and an ambient temperature of 22±2 °C. At 23 days of age, surgery was performed on all guinea pig pups under general anaesthesia induced by intramuscular injections of Ketamine hydrochloride (100 mg/kg body weight, Centaur laboratories, South Africa) and Xylazine (4 mg/kg body weight, Centaur laboratories, South Africa). A sterile wax-coated temperature-sensitive radiotelemeter (Mini-Mitter, Sunriver, OR, USA) was implanted into the abdominal cavity of each pup. The telemeters had a mass of approximately 3 g and previously had been calibrated in a water bath, over a range of biological temperatures, against a precision quartz-crystal thermometer (Quat 100, Heraeus, Germany) to an accuracy of 0.1 °C. The animals were allowed seven days to recover from surgery. During this time, the pups were separated from their mothers and placed into individual cages and fed a diet consisting of commercial rodent pellets. All animals had free access to water supplemented with vitamin C (500 mg/l).

Eight days after completion of the study, the guinea pigs were again anaesthetised, blood taken by cardiac puncture, and the animals killed by an intraperitoneal injection of an overdose of sodium pentobarbitone (Eutha-naze, Centaur laboratories, South Africa).

2.2. Experimental procedure

The guinea pigs pups were randomly divided into two groups with litter-mates split between groups. One group ($n=5$) received intramuscular (im) injections of 50 μg/kg body mass of the pyrogen, muramyl dipeptide (MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine, Sigma, USA) in a volume of 0.6 ml. The remaining animals comprised a control group which was injected im with the same volume of sterile normal saline.

All pups were given a series of eight injections spaced 4–5 days apart over an experimental period of about 30 days. On the days of injection, body temperature of each animal was measured every 15 min starting 1 h before injections and continuing for a further 6 h, using a hand-held wand receiver (RLA 3000 DataSciences International, MN, USA) connected to a Heath 2372 frequency counter multimeter (MiniMitter, Sunriver, OR, USA) which, when brought to the outside of the animal's cage, detected the output from the intra-abdominal radiotelemeter. All injections were made at 09:00.

Food intake was monitored daily, starting two days before the first injections were made, by determining food consumed and food spilled, subtracted from a weighed amount given to each animal each day.

Body weights of the animals were measured at 4-d intervals, out of phase with the injections of pyrogen or saline, using an electronic balance (Clover Mini-balance), and to an accuracy of 1 g.

At the termination of the experimental period, the blood sampled by cardiac puncture was centrifuged at 600 g for 10 min at 4 °C and the serum frozen at −70 °C until analysed for concentrations of triglyceride, total protein, iron, albumin, zinc and glucose. All analyses were carried out by the South African National Health Laboratory Service using standard medical laboratory techniques except the concentration of IGF-1 in serum, which was measured by immunoenzymometric assay (IEMA) using an OCTEIA IGF-1 KIT (AEC Amersham, South Africa).

2.3. Statistical analyses

Data are expressed as the mean±SD where appropriate and values of $P<0.05$ are considered to be statistically significant. One- or two-way repeated-measures analysis of variance (ANOVA) was used to detect differences within each group and the student's unpaired *t*-test, with Bonferroni correction when necessary, was used to detect differences between groups.

Six-hour fever indices (FI, in °C/h) were calculated as the time integral of change in abdominal temperature for 6 h after injection, using the average abdominal temperature measured for an hour before injection, as baseline.

Change in body mass (in g) is expressed as the difference in body weight between two consecutive weighings. The rate of weight gain is expressed as change in mass over four days (g per 4 d).

2.4. Ethics

The study was approved by the Animal Ethics Committee of the University of the Witwatersrand, South Africa (AESC number 98/99/4).

3. Results

3.1. Body temperature responses

Fig. 1 shows the changes in abdominal temperature of two guinea pig pups, one from each experimental group, after an intramuscular (im) injection of either sterile saline or 50 μg/kg MDP. The responses are typical of those in the experimental groups.

Injection of MDP resulted in a rise in body temperature commencing about 120 min after injection. The rise in abdominal temperature was about 1.5 °C above pre-injection levels for all pups. There was no significant increase in the abdominal temperatures of guinea pigs injected with sterile saline.

Fig. 2 shows the mean±SD of the six-hour fever index (FI) calculated for each group, for each day on which injections took place. On each occasion, injections of MDP produced a significantly higher mean FI compared to injections of sterile saline (unpaired *t*-test, $P<0.05$–0.0001) in the pups. All eight MDP injections produced significant rises in guinea pig body temperature of similar magnitude; there was no significant