The persistence of hepatitis B antigen in the bloodmeal of the potential medicinal leech, *Asiaticobdella buntonensis*

G. B. WILKEN, C. C. APPLETON

Abstract The persistence of the hepatitis B virus surface antigen (HBsAg) was used as an index of the survival time of this virus within the gastro-intestinal tract of the potential southern African medicinal leech, *Asiaticobdella buntonensis*. HBsAg was tested for in blood/faecal material at five intervals over 15 weeks. Samples from both the midgut and the rectum remained positive for the entire test period, although with decreasing strength. The results are compared with reports on other arthropods which indicate increasing antigen persistence with increasing body size. The findings implicate medicinal leeches as mechanical vectors of HBV and possibly of other medically important viruses, and argue against using leeches of suspect or unknown origin in the alleviation of venous congestion in failing microsurgical procedures.


Since the medicinal leech came back into use to alleviate venous congestion in failing microsurgical procedures, there have been several reports of leech-related nosocomial infections, with Aeromonas hydrophila the causative agent. Bacteriological investigation of the gut flora of leeches has been performed on Hirudo medicinalis and other bacteria, notably water-borne varieties, have been isolated, but not as yet implicated in leech-related infections. However, the presence of diverse gut flora in medicinal leeches and their occasional role in leech-associated infection reveal little about the risk of cross-infection between patients with infective agents associated with the previous bloodmeal. For this reason, and because of limited data on the fate of viruses in the bloodmeal of leeches, the persistence of hepatitis B surface antigen (HBsAg) in the bloodmeal of the leech, *Asiaticobdella buntonensis*, was investigated.

Hepatitis B virus (HBV) was chosen because its hardness makes it a likely candidate for transmission by leeches, and one against which the persistence of less robust viruses can be inferred. In addition, HBV's persistence and potential for transmission by various arthropods have been studied by several authors, making for interesting comparisons with regard to the persistence of this virus in sanguivorous leeches.

Material and methods

Leeches that weighed between 0,50 g and 1,0 g, housed under laboratory conditions (temperature 26 ± 1°C, 12:12 hours light/dark) without a bloodmeal for more than 4 months were fed HBV-infected blood through a membrane feeder until satiated. Blood was obtained from the Natal Blood Transfusion Services and its HBsAg-positivity confirmed by radio-immunooassay (RIA). Positivity was expressed using the positive ratio (specimen count/cut-off value). The leeches were divided into batches of five, and housed separately in plastic ice-cream containers covered with fine gauze netting.

A pilot study revealed that HBsAg remained strongly positive for more than 28 days. In the present study, batches were killed by freezing at intervals of 3, 6, 9, 12 and 15 weeks (21, 42, 63, 84 and 105 days) respectively so as to document the duration of the viral marker more completely. The gut was exposed by a mid-dorsal incision and swabs of blood/faecal material were taken from the midgut and the rectum. The swabs were eluted in 1% bovine serum albumin (BSA) (pH 7.0) and tested for HBsAg.

Results and discussion

The survival time of the HBsAg in the bloodmeal of *A. buntonensis* is shown in Table I. Because the gut contents of most of the leeches (48/50) remained positive for HBsAg as determined by RIA for the duration of the trial, the results were broken down according to the categories used by the Department of Virology at King Edward VIII Hospital, Durban. These are negative (-), weak positive (+), strong positive (+++) and very strong positive (+++++).
The fate of HBsAg in the bloodmeal of the leech A. buntunensis

<table>
<thead>
<tr>
<th>Batch</th>
<th>Days elapsed post feeding</th>
<th>Gut region investigated</th>
<th>RIA result</th>
<th>Overall positive (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>21</td>
<td>M</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>42</td>
<td>R</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>63</td>
<td>R</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>84</td>
<td>M</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>A</td>
<td>105</td>
<td>R</td>
<td>+</td>
<td>4</td>
</tr>
</tbody>
</table>

- negative; ~ weak positive; ++ = strong positive; +++ = very strong positive; M = midgut; R = rectum.

The occurrence of +++ results for swabs taken from the midgut region in the absence of the same from the rectal fluids, probably do not reflect differences in the digestive processes or gut morphology. The blood and its breakdown residuals reach the rectum long after ingestion, and rectal swabs taken soon after feeding are comprised largely of the remnants of the previous uninfected bloodmeal.

The presence of the HBsAg marker is all that need remain intact for a sample to test positive by RIA. Thus their occurrence in blood that is not freshly obtained from a patient, and therefore not subject to continual removal of antigens by the immune system, may well contain intact antigenic fragments that reveal little about the presence, viability or replicative activity of the pathogen. Caution must therefore be exercised in interpreting positive RIA readings from invertebrate bloodmeals as indicative of the presence of viable viruses. This is particularly so where there is no evidence of viral replication, and antigenic persistence is correlated with the digestion and excretion of the bloodmeal, as is the case with A. buntunensis. In the case of a voluminous bloodmeal that remains in the leech for extended periods with little putrefaction or active digestion, the persistence of antigen markers for protracted periods gives only an indication of the gastric conditions to which HBV or any other virus would be exposed. The results indicate a benign environment in which many medically important viruses could survive.

Although leeches have been discounted as biological carriers of viruses, they have been found to harbouring of this virus in its bloodmeal. This, and the evidence of HBV transmission by certain arthropods, argue very strongly against using leeches of unknown or suspect origin in surgery and support the widely accepted principle of avoiding any situation that could effect leech-mediated cross-infection in the clinical context.

We gratefully acknowledge the assistance of Dr I. M. Windsor and the staff of the Department of Virology, University of Natal Medical School.

The research formed part of a study funded by the South African Medical Research Council postgraduate scholarship.

REFERENCES

Maternal nicotine exposure during pregnancy and development of emphysema-like damage in the offspring

G.S. MARITZ, K. M. WOOLWARD, G. DU TOIT

Abstract

The aim of this investigation was to determine whether nicotine exposure (1 mg/kg body mass/d) during pregnancy and lactation contributes to the rupturing of alveolar septa in the lungs of neonatal rats. These rats received nicotine only via the placenta and mother’s milk. The results show that maternal nicotine exposure interferes with elastic tissue formation. It also interferes with alveoli formation and causes the development of emphysema-like lesions. It is therefore suggested that maternal nicotine intake from smoking during pregnancy and lactation may interfere with lung development and maturation to an extent that increases susceptibility to emphysema.

Early postnatal morphogenesis of the rat lung is characterised by a number of qualitative tissue, cellular, sub-cellular, and molecular remodellings. The developmental events contribute in different ways to the transformation of an immature lung into a structurally and functionally competent organ. Interference with the process of lung development may thus have an adverse influence on the metabolic, structural and functional development of the lung, its effectiveness and resistance against disease.

A study by Collins et al. demonstrated that maternal smoking adversely modifies fetal lung growth. It is not known which of the many components of cigarette smoke is responsible for this. However, Luck and Nau clearly illustrated that maternal smoking resulted in the accumulation of considerable amounts of nicotine in fetal blood and mother’s milk. Since nicotine is rapidly absorbed by the infant and since it accumulates in the respiratory tract after absorption, it is possible that this alkaloid may have a detrimental effect on lung growth and development. Maritz found that maternal nicotine exposure during pregnancy had an irreversible adverse effect on carbohydrate metabolism in the neonatal rat lung. He also showed enhanced cellular multiplication in the lungs of these rat pups. Recently Maritz and Woolward demonstrated a change in the type I/type II cell ratio from 1:58:1 for normal rat pups to 0.22:1 for the nicotine-exposed rat pups, as well as thickening of the blood-air barrier in the latter group.

Smoking is a major risk factor associated with the development of emphysema, a disease characterised by erosion of the alveolar walls. This erosion is the result of deterioration of the components of the lung connective tissue framework and changes in the composition of the ground substance responsible for the stability of this framework. Since nicotine interferes with elastogenesis and other metabolic pathways of the neonatal lung, as well as with the cellular development thereof, it is possible that maternal nicotine exposure may induce a sequence of events which causes emphysema. The aim of this study was to determine whether maternal nicotine exposure during pregnancy and lactation, in doses comparable with the intake of habitual smokers, does induce emphysema-like lesions in the lungs of the offspring.

Materials and methods

Animals

White virgin female rats (Wistar descendants) of 200 - 250 g were used in the present investigation and were fed a stock diet (Epol rat cubes) throughout the experiment. All animals received food and tap water as required. Room temperature was kept at 22°C and day-night cycle of 12 hours was maintained. We maintained our own breeding programme for both control and experimental animals. The length of gestation averaged 22.5 days. Animals were mated overnight and were afterwards randomly assigned to control and experimental groups. Whether mating had occurred was determined by the presence of mating plugs and sperm in vaginal smears. The day of the appearance of the vaginal plug was designated day 0 of gestation. Nicotine exposure commenced on day 7 of gestation to avoid nicotine interference with blastocyte implantation and initial embryonic growth; it was continued until the pups were weaned 3 weeks after birth. The dams received single daily doses of 1 mg nicotine/kg body weight subcuta-