THE EFFECTS OF *TRICHINELLA SPIRALIS* INFECTION ON RENAL FUNCTION IN RATS

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Abstract. *Trichinella spiralis* infection was induced in rats by oral feeding of infective larvae. Four weeks later, renal function, including renal plasma flow (RPF), glomerular filtration rate (GFR), excretion rate of protein, sodium and potassium were determined using clearance technics. There were no significant changes in these parameters. However, plasma urea nitrogen was significantly higher in the infected group, suggesting that either an impaired regulation of renal tubular urea transport or an increased skeletal muscle breakdown is likely.

INTRODUCTION

Parasitic nephropathies has been identified over many years. These include glomerular, microvascular, tubulo-interstitial and lower urinary lesions (Goldman and Lambert, 1985; Barsoum, 1997). Trichinosic nephritis was first characterized by inflammatory infiltrations and dystrophic alterations of tubular epithelium (Pambuccian and Cironeanu, 1961). Distinct opacity of the kidney cortex and swelling of intracapsular, intratubular and interstitial location were observed in man (Gould, 1970). Focal hemorrhages and infarction were also seen. In addition, mild or transient proteinuria was reported to be associated in trichinosis (Barsoum, 1997). The etiology of these manifested lesions and changes has not been yet demonstrated. The degree of kidney damage may depend on several factors such as severity of the infection and/or susceptibility of the host and duration of the infection. The purpose of this study was to examine the kidney functions of *Trichinella spiralis* infected rats 4 weeks after infection by determining renal plasma flow (RPF), glomerular filtration rate (GFR) and urinary excretion of protein, sodium and potassium. Plasma urea nitrogen concentration was also measured. The degree of infection was designed using sublethal dose of larvae.

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MATERIALS AND METHODS

Animals

Male Wistar rats, obtained from the National Animal Center, Nakhon Pathom, Thailand were used in all experiments.

Trichinella spiralis infection procedure

Induction of infection was performed at the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University. The methods were, in brief, as follows. Two month-old rats were each orally fed with 300 infective larval stage of *T. spiralis.* The parasite was originally isolated from patients during a trichinosis outbreak in Mae Hong Son Province in 1986 and has been transferred and maintained in laboratory mice at the department thereafter.

Experimental protocol

Renal functions were determined in both control and in *T. spiralis* infected rats. After four weeks of the infection, renal function including RPF and GFR were performed by measuring clearances of para-aminohippuric acid (PAH) and polyfructosan (PFS). The clearances of PAH and PFS are the indices of RPF and GFR, respectively (Smith *et al*, 1945; Berglund, 1965). Urinary excretion of protein, sodium and potassium were also performed. After completion of clearance experiment, plasma urea nitrogen concentration (BUN) was measured and the diaphragm of infected rat was cut, pressed between two glass slides and examined under

stereomicroscope to confirm the infection.

Animal preparation for clearance study

Anesthetization was induced by intraperitoneal (i.p.) injection with Inactin (sodium salt of ethyl-1-methyl-propyl malonyl-thiourea, RBI, USA) at a dose of 110 mg/kg body weight (BW) and an additional dose was given if necessary to maintain anesthesia during the experiment. The animal was then placed on a heated operating table that maintain body temperature at 37ºC via rectal thermistor feedback system. Tracheostomy was performed using a short piece of polyethylene tubing in order to aspirate the secretion that might block the airway under anesthesia. The right carotid artery was canulated with polyethylene tubing for measuring arterial blood pressure (ABP) and for blood sampling during the course of experiment. ABP was monitored with a pressure transducer (Statham PE23DE) and recorded on a Grass Polygraph (Model 7D) recorder. The left jugular vein was canulated with polyethylene tubing. Intravenous infusion of clearance markers including 1% para-aminohippuric acid (PAH) and 8% polyfructosan (PFS) was kept maintained at the rate of 0.8 ml/hour/100g BW by infusion pump (Model A975, Harvard Apparatus). The concentration of PAH and PFS were calculated in order to maintain plasma level of both substances suitable for determination of renal plasma flow (RPF) and glomerular filtration rate (GFR). The urinary bladder was exposed by a suprapubic incision and canulated with a flanged canula for urine collection. Urine volume was determined gravimetrically in a preweighed cup assuming a density of 1g/ml. Clearance measurements were performed on both kidneys for 3 hours. At the end of experiment, a large volume blood sample was collected and plasma was separated for blood urea nitrogen (BUN) determination. The animal was then sacrificed and both kidneys were excised, decapsulated and weighed.

Analytical methods

PAH concentration was seasured by the method of Smith *et al* (1945), PFS concentration in urine and plasma samples was measured by the anthrene method (Davidson and Sackner, 1963), and sodium and potassium concentrations in both urine and plasma were analyzed by AVL electrolyte analyzer (Model 988-3). Blood urea nitrogen (BUN) was determined by a diacetyl monoxime method (Wybenga *et al,* 1971). Total protein was determined with the method of Lowry *et al* (1951).

Calculations

Calculations of each parameter were performed as follows:

Urine flow rate (V) = urine volume/collection time (µl/min/g kidney weight)

Clearances (C_X) of PFS, PAH, Na^+ and K^+

$C_X = U_X \times V/P_X$

whereas
C_X = clearance of X
U_X = urine concentration of X
V = urine flow rate
P_X = plasma concentration of X

The clearance of PAH is an index of effective renal plasma flow. The actual renal plasma flow was calculated assuming a 90% extraction of PAH. The clearance of PFS has been used as a measurement of GFR in rats and its renal clearance does not differ significantly from inulin clearance (Berglund, 1965).

Statistical analysis

All clearance data are presented as average values of six periods of thirty minute urine collection and expressed as mean ± SEM. Student's unpaired *t*-test was used to compare mean values between control and infected group. A p-value less than 0.05 was considered significant difference.

RESULTS

Comparative clearance studies of control and *T. spiralis* infected groups are shown in Table 1. When compared with controls, a four-week infected rats showed no significant changes in RPF, GFR, urinary excretion of sodium, potassium and protein. Plasma concentration of sodium and potassium were also unaltered. It is noted that the infected group tended to have higher GFR with lower sodium excretion rate ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) when compared with controls. This may suggest a tendency of impaired glomerulo-tubular balance. However, plasma urea nitrogen was significantly higher in the infected group (17.4 ± 2.7mg%) compared with controls (10.7 ± 0.8mg%) ($p<0.05$). Other parameters measured during the clearance studying period, *ie* mean arterial blood pressure, hematocrit, plasma concentration of PFS and PAH were not significantly different.

Table 1
Clearance studies of control and *Trichinella spiralis* infected rats.

	Control	*T. spiralis* infected
Number of rat	10	8
Body weight (g)	289 ± 8	280 ± 16
Kidney weight (g/100g BW)	0.70 ± 0.02	0.64 ± 0.02
Hematocrit (%)	47.2 ± 1.2	45.4 ± 1.5
Mean arterial pressure (mmHg)	134 ± 5	127 ± 2
Urine flow rate (μl/minute/g KW)	15.0 ± 2.9	10.6 ± 1.9
P_{PFS} (mg%)	103.8 ± 2.8	100.6 ± 5.4
GFR (ml/minute/g KW)	1.49 ± 0.07	1.99 ± 0.44
P_{PAH} (mg%)	1.29 ± 0.11	0.94 ± 0.07
RPF (ml/minute/g KW)	4.79 ± 1.02	4.56 ± 0.75
P_{Na} (mmol/l)	141.9 ± 1.8	140.4 ± 4.4
$U_{Na}V$ (mmol/minute/g KW)	6.06 ± 1.7	2.56 ± 0.6
FE_{Na} (%)	3.02 ± 0.9	1.08 ± 0.3
P_K (mmol/l)	3.75 ± 0.08	4.03 ± 0.10
U_KV (mmol/minute/g KW)	0.97 ± 0.14	1.22 ± 0.16
FE_K (%)	19.6 ± 3.1	17.9 ± 2.7
Blood urea nitrogen (mg%) (n)	10.7 ± 0.8	17.4 ± 2.7[a]
Total urinary protein (mg/ml)	0.82 ± 0.28	0.67 ± 0.14
Protein excretion (mg/minute/g KW)	23.5 ± 4.8	20.6 ± 5.8

Data are means ± S.E.M.
[a]$p<0.05$, (Student's unpaired *t*- test compared with control).
BW = body weight, KW = kidney weight, P_{PFS} = plasma concentration of polyfructosan, , P_{PAH} = plasma concentration of PAH, P_{Na} = plasma concentration of sodium, P_K = plasma concentration of potassium, GFR = glomerular filtration rate, RPF = renal plasma flow, $U_{Na}V$ = sodium excretion rate, U_KV = potassium excretion rate, FE_{Na} = fractional sodium excretion, FE_K = fractional potassium excretion.

DISCUSSION

The concentration of urea in plasma, reported as blood urea nitrogen (BUN), was 63% significantly higher in the infected rat group compared with controls. The BUN value depends on the rate of urea production as well as on GFR. Urea production is increased by protein loading (dietary or due to the protein content of the blood in gastrointestinal hemorrhage) and catabolic states in which there is an increase in muscle protein catabolism (Marsh and Knepper, 1992). Beckett (1961) reported a decrease in protein content of trichinous muscle. In this case, it is likely that the higher BUN concentration found in the 4 week infected rats may be due to the higher muscle protein breakdown.

Using protein determination by Lowry's method, urinary total protein excretion rate of our control male rat was as same as reported earlier (Alt *et al*, 1985). Urinary protein concentration and protein excretion rate of the 4 week *T. spiralis* infected rats were not different from controls as shown in Table 1. In patients, Barsoum (1997) reported that proteinuria may be observed in association with trichinosis. It has been suggested that urinary protein excretion may be increased in the earlier stage of infection or during the encapsulation process of the parasite. At this stage, fever, edema of soft tissues and muscle pain and fatigue were also seen. However, this study was not to demonstrate an increase in protein excretion. This may be because of the species difference of the host which in turn made the differences in the susceptibility of parasite and the duration of symptom development.

T. spiralis infected rats did not appear to exhibit impaired kidney function. Besides an unaltered protein excretion rate, RPF, GFR, plasma sodium and potassium concentration, and sodium and potassium excretion remained unchanged. However, it is noted that the GFR of *Trichinella*

infected rats was 34%, insignificantly higher than controls. Since GFR depends on net filtration pressure (NFP) and filtration coefficient (K_f), it is suggested that the infection may be, in part, responsible for the increased GFR by increasing K_f rather than NFP. This is because mean arterial blood pressure and RBF did not change much when compared to GFR. In addition, sodium excretion rate of the infected rats was 58% lower than in controls even though this value was not statistically different. While GFR has a tendency to be increased in 4 weeks *T. spiralis* infection, on the other hand, sodium excretion rate tends to be decreased. It is concluded an impaired tubulo-glomerular balance might occur and this may lead to a manifest tubuloglomerular nephritis (trichinosic nephritis) although further experimental design and data are needed to confirm this statistically.

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