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ORIGINAL ARTICLE

An atherogenic lipid profile with low serum paraoxonase-1 activity during nematode infection in rats

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ABSTRACT

Background Inflammation and oxidative stress are associated with cardiovascular diseases and underlying atherosclerosis. The high density lipoprotein (HDL)-associated paraoxonase-1 (PON1) enzyme is known to be involved in the protection of serum lipids from such oxidation. Nonetheless, the disturbances of lipid profile during nematode-infected model have not yet been studied. Therefore, we aimed to explore the effects of *Nippostrongylus brasiliensis* infection in male Wistar rats, a model of human gastrointestinal nematode infections, on hepatic PON1 synthesis and the levels of lipid parameters.

Materials and methods *Nippostrongylus brasiliensis*-infected rats fed standard and high-fat diets. Serum paraoxonase and arylesterase activities were measured on day 0, 2, 4, 7, and 14 post-infection (PI). Hepatic PONs and pro-inflammatory cytokines mRNA expression levels were evaluated in a standard diet-fed groups, and the disturbances in lipid profile as well as the levels of thiobarbituric acid reactive species (TBARS) and oxidized-LDL (Ox-LDL) were measured in high-fat diet-fed groups.

Results We found that *N. brasiliensis*-infected rats fed the standard diet show a significant reduction in serum PON1 activity and down-regulation of hepatic PON1 mRNA expression as well as up-regulation of hepatic IL-1β, IL-β receptor (R), TNF-α, and TNFR1 mRNA expressions in association with hepatic recruitments of Kupffer cells and neutrohils. In the presence of the high-fat diet, *N. brasiliensis* infection increases serum triglycerides, total cholesterol, LDL/VLDL, TBARS and Ox-LDL as well as decreases serum HDL coinciding with a maximum serum PON1 reduction.

Conclusions *Nippostrongylus brasiliensis* infection can induce atherogenic lipid profile and reduce serum PON1 activity.

Keywords Atherosclerosis, high-fat diet, lipid metabolism, *Nippostrongylus brasiliensis*, oxidized-LDL, paraoxonase-1 reduction.

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Introduction

Paraoxonase-1 (PON1) is a member of a family of proteins that also includes PON2 and PON3, the genes for which are clustered in tandem on the long arms of human chromosome (q21·22) [1]. Serum PON1 is predominantly synthesized by hepatocytes and released into the circulation, where it resides principally on high density lipoprotein (HDL) particles [2] and is believed to contribute to the atheroprotective effect of HDL [3]. Although other HDL-associated enzymes, such as lecithin cholesterol acyltransferase LCAT and apo AI, may contribute to anti-oxidant effects of HDL, HDL-associated PON1 was primarily responsible for the anti-oxidant/anti-inflammatory functions of HDL [4].

According to the 'oxidative modification hypothesis', atherogenesis is initiated by oxidation of the low-density lipoprotein (LDL), and the process of atherosclerosis can be significantly ameliorated by the presence of a variety of antioxidant compounds [5]. In support of these findings, several human studies have shown an inverse linear relationship

between the concentration of oxidized-LDL (Ox-LDL) in the circulation and PON1 activity, strongly implicating PON1 in the metabolism of Ox-LDL *in vivo* [6].

On the other hand, the concentration and mainly the activity of PON1 can be modified by several factors [1]. Recently, the authors have found that *Nippostrongylus brasiliensis* infection, a model of gastrointestinal (GI) nematode infection, markedly decreases serum PON1 (paraoxonase and arylesterase) activity in Wistar rats in association with inflammation and increased serum levels of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) [7]. With regard to human parasitic infections, GI nematodes are one of the most common causes of chronic infections, particularly in the developing world where approximately 50% of the population suffers from infections with one or more worm species causing substantial morbidity and hundreds of thousands of deaths [8,9].

At the same time, atherosclerosis is a serious health epidemic in developed countries, and its rising prevalence in developing nations suggests that it will become the chief cause of morbidity and mortality [10,11]. Projections by the World Health Organization (WHO) suggest that by 2025, about three quarters of the estimated 1·2 billion people aged 60 years and older will reside in developing countries [12], and there is an elevated risk that such individuals could develop atherosclerosis.

Therefore, we attempted to study the possible impact of our findings of reduced serum PON1 activity during *N. brasiliensis* infection, a model of human nematode infection, on lipid metabolism by using *in vivo* normal and high-fat diet-fed rat models.

Materials and methods

Experimental animals

Thirty-five male Wistar rats were purchased from Charles River Japan (Yokohama, Japan) for use in this study. The animals were housed in clean cages and had access to diet and clean water *ad libitum*. The environment was controlled in terms of light (12:12-h light-dark cycle starting at 8:00 AM) and room temperature (23 ± 3 °C). All protocols were approved by the University of Miyazaki institutional review board for animal experiments.

Parasitological techniques

The strain of *N. brasiliensis* used in this study was maintained in our laboratory by serial passage in Wistar rats using subcutaneous inoculation of 3000–4000 third-stage larvae (L3) prepared by the charcoal culture method [13]. Experimental rats were infected with L3 of *N. brasiliensis* by subcutaneous inoculation into the flank region. Infection was confirmed by counting faecal egg output as eggs per day.

Serum PON1 activity

Serum PON1 activity was assayed as described by Beltowski *et al.* [14] using two synthetic substrates; paraoxon (diethyl-*p*-nitrophenyl phosphate; Sigma Chemical Co., St. Louis, MO, USA) and phenyl acetate (Nacalai Tesque Inc., Kyoto, Japan). Both activities were measured at 25 °C.

Determination of parameters of lipid metabolism

Serum samples were used to measure triglycerides [15] (Cayman Chemical Company, Ann Arbor, MI, USA), total cholesterol, HDL and LDL/VLDL [16] (BioVision Inc., Mountain View, CA,USA), while Ox-LDL in heparinized plasma was measured using oxidized LDL competitive Enzyme-linked immunosorbent assay (ELISA) [17] (Mercodia, Uppsala, Sweden).

Measurement of serum TBARS levels

Levels of thiobarbituric acid reactive substance (TBARS) expressed in terms of malondialdehyde (MDA), as an index of serum lipid peroxides, were measured in serum using a TBARS assay kit [18] (Cayman Chemical Company).

Analysis of mRNA expression of hepatic PONs (PON1, PON2 and PON3) and cytokines genes using real time-PCR

To better understand the effects of *N. brasiliensis* infection on hepatic inflammation and its relationship with PON synthesis, expression of hepatic PON (PON1, PON2 and PON3) genes and various cytokines (interleukin (IL)-1β, IL-1β receptor (R), IL-6, IL-6R, TNF-α, TNFR1 and TNFR2) genes were analysed by real time-PCR using sense and anti-sense primers.

Total cellular RNA was extracted from liver tissue using an RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Real-time PCR was performed using a Power SYBR Green RNA-to-C_t 1-step kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

PCR was performed in a 10-μl reaction volume containing 0·08 μl of RT enzyme mix (125 ×), 5 μl of RT-PCR mix (2 ×), 3 μl of primers (sense and anti-sense) (33·3 nM), 1 μl of each RNA template (0·1 pg/μl) and 0·92 μl of nuclease-free water. Primers sets were as follows: PON1, sense (5′-AAG TAT GTC TAT ATC GCT GAA TTG C-3′) and anti-sense (5′-CAC AGG ATC CAC AGA GAT GTT ATC-3′); PON2, sense (5′-CTA ACG GCC AGA AGC TCT TCG-3′) and anti-sense (5′-GAT GTA CAC TGT CGT CAC CGA T-3′); PON3 sense (5′-ACT CCA GTG AAG GTA ATT CAG C-3′) and anti-sense (5′- GAT CCT CCG GGT TAT AGA TCA AG-3′); IL-1β, sense (5′-CAC CTC TCA AGC AGA GCA CAG-3′) and anti-sense (5′-GGG TTC CAT GGT GAA GTC AAC-3′); IL-1βR, sense (5′-GTT TTT GGA

ACA CCC TTC AGC C-3′) and anti-sense (5′-ACG AAG CAG ATG AAC GGA TAG C-3′); IL-6, sense (5′-TCC TAC CCC AAC TTC CAA TGC TC-3′) and anti-sense (5′-TTG GAT GGT CTT GGT CCT TAG CC-3′); IL-6R, sense (5′-AAG CAG GTC CAG CCA CAA TGT AG-3′) and anti-sense (5′-CCA ACT GAC TTT GAG CCA ACG AG-3′); TNF-α, sense (5′-AAA TGG GCT CCC TCT CAT CAG TTC-3′) and anti-sense (5′-TCT GCT TGG TTT GCT ACG AC-3′); TNFR1, sense (5′-TTG TAG GGA TTC AGC TCC TGT C-3′) and anti-sense (5′-CTC TTA CAG GTG GCA CGA AGT T-3′); TNFR2, sense (5′-TGC AAC AAG ACT TCA GAC ACC GTG-3′) and anti-sense (5′-AGG CAT GTA TGC AGA TGG TTC CAG-3′); and 18S rRNA, sense (5′-GAG GTG AAA TTC TTG GAC CGG-3′) and anti-sense (5′-CGA ACC TCC GAC TTT CGT TCT-3′).

The real-time-PCR cycling program consisted of reverse transcription at 48 °C for 30 min, initial PCR activation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and a dissociation curve was added to the protocol whenever necessary. Real-time-PCR assay was performed using a 7300 real-time-PCR system (Applied Biosystems).

Changes in gene expression were calculated from the obtained cycle threshold (C_t) values provided by real-time PCR instrumentation using the $2^{-\Delta\Delta Ct}$ calculation, where ΔC_t indicates the C_t changes in target genes in comparison with a reference (house-keeping) gene (18S rRNA) [19].

Histopathological analysis

The right lobes of the liver were collected from control and *N. brasiliensis*-infected rats fed standard diet and were fixed in neutral buffered formalin (pH 7·2) (Nacalai Tesque Inc., Kyoto, Japan). To quantify the number of Kupffer cells, sections were stained with methyl green pyronin stain, and cell numbers were counted in 10 randomly selected fields at a magnification of ×400. To quantify the number of neutrophils, sections from each specimen were stained with haematoxylin and eosin.

Diet and experimental design

Experiment 1. This experiment was performed to examine the mechanisms of PON1 reduction during *N. brasiliensis* infection by evaluating hepatic PONs and cytokines mRNA expressions at various time points following infection. A total of 25 male Wistar rats (75–85 g) aged 4 weeks were used in this experiment. After 1 week of acclimatization, rats were randomly allocated into five groups of five rats each and fed a standard diet. Standard diet consisted of 10·67% lipids, 22·85% proteins and 66·48% carbohydrates (kcal) with 0·025% cholesterol content (Nosan, Yokohama, Japan). Rats in the experimental groups [days 2, 4, 7 and 14 post-infection (p.i.)] were infected with 4000 L3 *N. brasiliensis*, while the control group (day 0) received only saline. At the indicated time points, serum, liver tissue in RNA*later* solution (kept at −35 °C until RNA isolation) and liver tissue in neutral buffered formalin (pH 7·2) were collected.

Experiment 2. This experiment was performed to evaluate the effects of serum PON1 reduction during *N. brasiliensis* infection on TBARS, lipid profiles and Ox-LDL levels. Ten male Wistar rats (45–55 g) aged 3 weeks were randomly allocated into two groups of five rats each. The groups were as follows: (i) control group and (ii) *N. brasiliensis*-infected group (4000 L3). Both groups received a high-fat diet immediately after arriving and for 2 weeks before infection with *N. brasiliensis*. The high-fat diet consisted of 82% lipids, 15% proteins and 3% carbohydrates (kcal) with 0·08% cholesterol content (KPT Oriental, Torisu, Japan) [20]. Blood for serum and heparinized plasma samples was collected from the jugular vein on days 0, 2, 4, 7 and 14 p.i. from the infected and control groups.

Statistics

Statistical analysis was performed using the statistical software package SPSS for Windows (version 16·0; SPSS Inc., Chicago, IL, USA). Student's *t*-test was used to determine significant differences between *N. brasiliensis*-infected groups and controls with regard to Kupffer and neutrophil cell numbers and hepatic gene expression. For evaluating the significance of differences in lipid parameters and TBARS, nonparametric *Kruskal–Wallis H* test was used to test differences among groups, after which differences between individual groups were evaluated by *Mann–Whitney U*-test. Nonparametric *Mann–Whitney U*-test was also used to evaluate the significance of differences in PON1 (paraoxonase and arylesterase) activity. Results are expressed as means ± standard error of the mean (SEM). *P* values less than 0·05 were considered to be significant.

Results

Experiment 1 (standard diet)

PON1 activity. Serum PON1 activities against paraoxon (paraoxonase) and phenyl acetate (arylesterase) are shown in Fig. 1a,b. A 27% reduction in paraoxonase activity ($P < 0·05$) was observed on day 2 p.i., while a 38% reduction ($P < 0·01$) was observed on day 7 p.i. Days 4 and 14 p.i. showed non-significant changes in paraoxonase activity (Fig. 1a), while significant reductions of 27% ($P < 0·01$), 19% ($P < 0·05$), 36% ($P < 0·01$) and 14% ($P < 0·05$) in arylesterase activity on days 2, 4, 7 and 14 p.i. respectively are illustrated in Fig. 1b.

TBARS levels during *N. brasiliensis* infection. Non-significant changes in serum TBARS levels at the various time points following infection with *N. brasiliensis* in rats fed standard diet are shown in Fig. 2a.

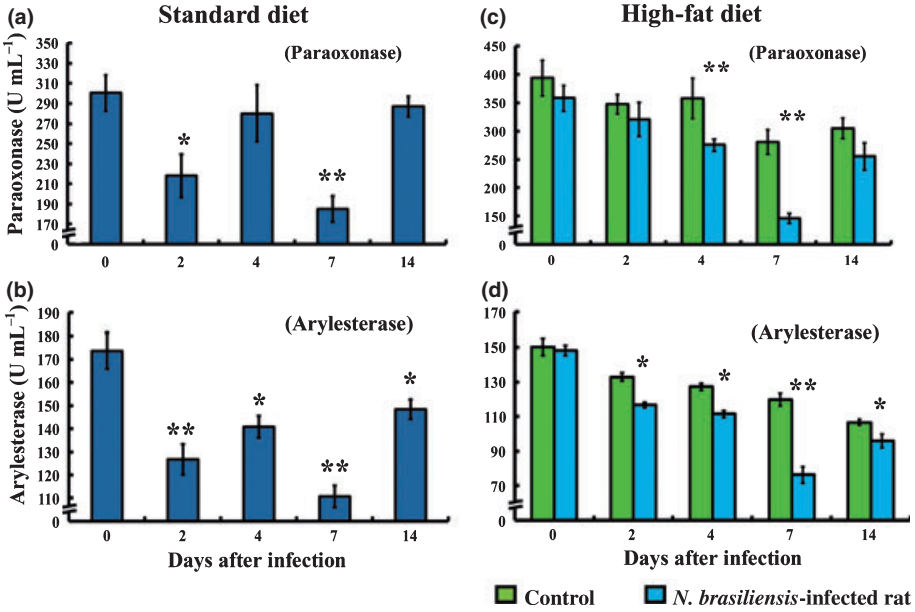

Figure 1 Effect of *N. brasilienis* infection on PON1 (paraoxonase and arylesterase) activity in male Wistar rats. Animals were infected with 4000 L3 of *N. brasiliensis* and fed standard (a,b) or a high-fat diet (c,d) then serum assays of PON1 activities were performed on days 0, 2, 4, 7 and 14 p.i. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, compared with control values. Bars represent means ± SEM (n = 3–5).

Changes in hepatic PON and cytokine mRNA expression following *N. brasiliensis* infection. Infection with *N. brasiliensis* induced significant down-regulation in hepatic PON1 mRNA on days 2 ($P < 0{\cdot}05$) and 7 ($P < 0{\cdot}01$) p.i. without significant changes in the hepatic expression of PON2 and PON3 (Fig. 4a–c). At the same time, *N. brasiliensis* infection induced significant ($P < 0{\cdot}05$) up-regulation of hepatic expression of IL-1β on day 2 p.i., significant ($P < 0{\cdot}05$) up-regulation of hepatic expression of IL-1βR on day 4 p.i., and non-significant changes in expression levels of IL-6 and IL-6R (Fig. 4d–g). Infection of rats with *N. brasiliensis* also induced significant up-regulation of TNF-α on days 4 ($P < 0{\cdot}05$) and 14 p.i. ($P < 0{\cdot}01$) and TNFR1 on day 7 p.i. ($P < 0{\cdot}01$) (Fig. 4h,i). There were non-significant changes in hepatic expression of TNFR2 (Fig. 4j).

Hepatic neutrophil and Kupffer cell numbers during *N. brasiliensis* infection. To identify the correlation between changes in hepatic functions manifested by reduced serum PON1 activity and the hepatic innate immune response, we counted the number of Kupffer cells and hepatic neutrophils in liver sections. The results indicated that the number of Kupffer cells gradually and significantly increased after infection with *N. brasiliensis* on days 2 ($P < 0{\cdot}05$), 4 ($P < 0{\cdot}01$), 7 ($P < 0{\cdot}001$) and 14 ($P < 0{\cdot}001$) p.i. (Fig. 5a,b). Similarly, hepatic neutrophil numbers were significantly higher ($P < 0{\cdot}001$) on days 2, 4, 7 and 14 following infection with *N. brasiliensis* (Fig. 5c,d).

Experiment 2 (high-fat diet)

PON1 activity. Serum paraoxonase activity showed 23% ($P < 0{\cdot}01$) and 48% ($P < 0{\cdot}01$) reductions on day 4 and 7 p.i., respectively (Fig. 1c). At the same time, arylesterase activity showed 12% ($P < 0{\cdot}05$), 12% ($P < 0{\cdot}05$), 36% ($P < 0{\cdot}01$) and 10% ($P < 0{\cdot}05$) reductions on days 2, 4, 7 and 14 p.i., respectively (Fig. 1d).

TBARS levels during *N. brasiliensis* infection. TBARS levels, expressed in terms of MDA, during infection with *N. brasiliensis* in rats fed high-fat diet showed a significant increase ($P < 0{\cdot}05$) on day 7 p.i., when compared with controls (Fig. 2b).

Lipid profile changes during *N. brasiliensis* infection in rats fed high-fat diet. We next examined plasma lipid levels during infection with *N. brasiliensis*. We found a significant increase ($P < 0{\cdot}05$) in triglyceride levels on day 7 p.i., when compared with days 0, 2, 4 and 14 p.i. (Fig. 3a). In addition, total cholesterol and LDL/VLDL levels showed significant increases ($P < 0{\cdot}05$) on day 7 p.i., compared with days 0, 2, 4

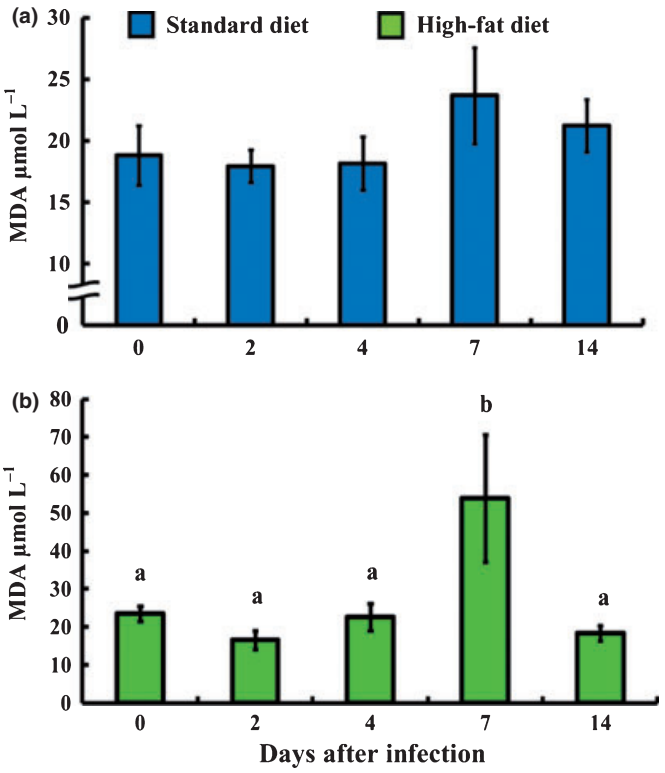

Figure 2 Serum concentration of thiobarbituric acid reactive substance (TBARS) expressed in terms of malondialdehyde (MDA) in male Wistar rats. MDA levels following infection with *N. brasiliensis* (4000 L3) in rats fed a standard (a) and a high-fat diet (b) on days 0, 2, 4, 7 and 14 p.i. (b) represents a significant value ($P < 0{\cdot}05$), compared with (a). Bars represent means ± SEM. ($n = 5$).

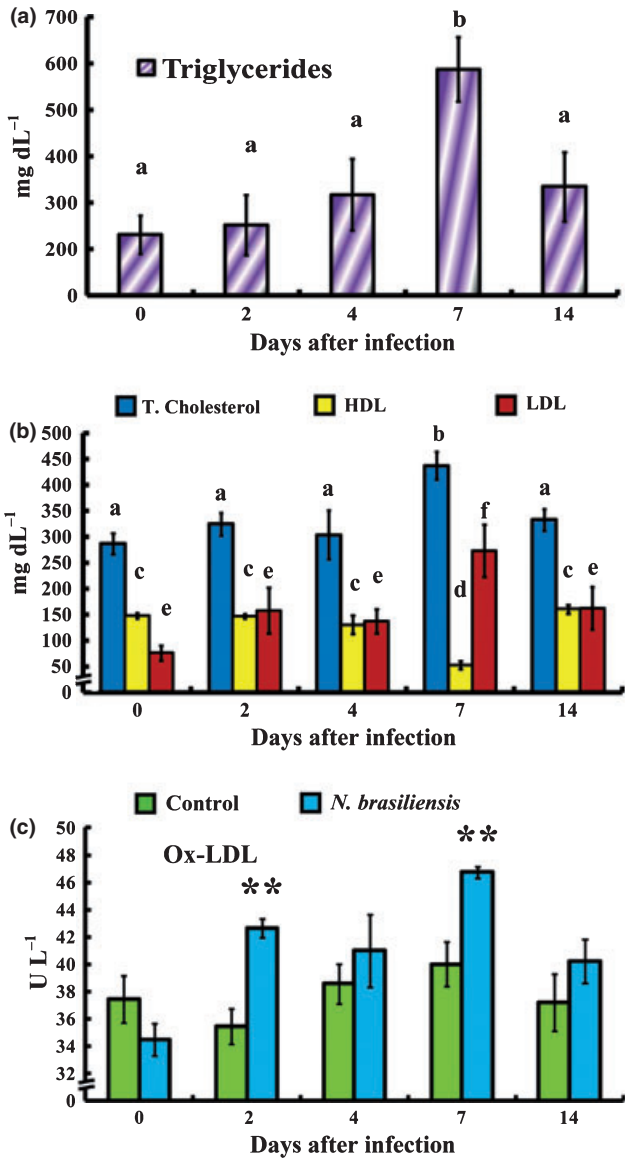

Figure 3 Serum levels of lipid metabolism in male Wistar rats following infection with *N. brasiliensis* (4000 L3). Triglycerides (a), total cholesterol, HDL and LDL/VLDL (b) oxidized-LDL (Ox-LDL) (c) levels in rats infected with *N. brasiliensis* fed a high-fat diet. (b) represents a significant value ($P < 0{\cdot}05$), compared with (a); (d) represents a significant value ($P < 0{\cdot}05$), compared with (c); (f) represents a significant value ($P < 0{\cdot}05$), compared with (e), $^{**}P < 0{\cdot}01$. Bars represent means ± SEM. ($n = 5$).

and 14 p.i. (Fig. 3b). By contrast, HDL levels showed significant decreases ($P < 0{\cdot}05$) on day 7 p.i, compared with days 0, 2, 4 and 14 p.i. (Fig. 3b). We also noted significant increases in Ox-LDL levels on days 2 and 7 p.i. ($P < 0{\cdot}01$), compared with control values (Fig. 3c).

Discussion

Atherosclerosis is an important underlying pathology of cardiovascular diseases and is the leading cause of morbidity and mortality in many countries. According to WHO estimates, by the end of next decade non-communicable diseases, including atherosclerosis, will account for approximately three quarters of all deaths in the developing world [21]. The previous studies have shown that PON1 has important roles in protecting LDL against oxidation, reversing the biological effects of oxidized LDL, and preserve the function of HDL by inhibiting its oxidation [22,23]. These results indicate the potential for PON1 to protect against atherogensis [4].

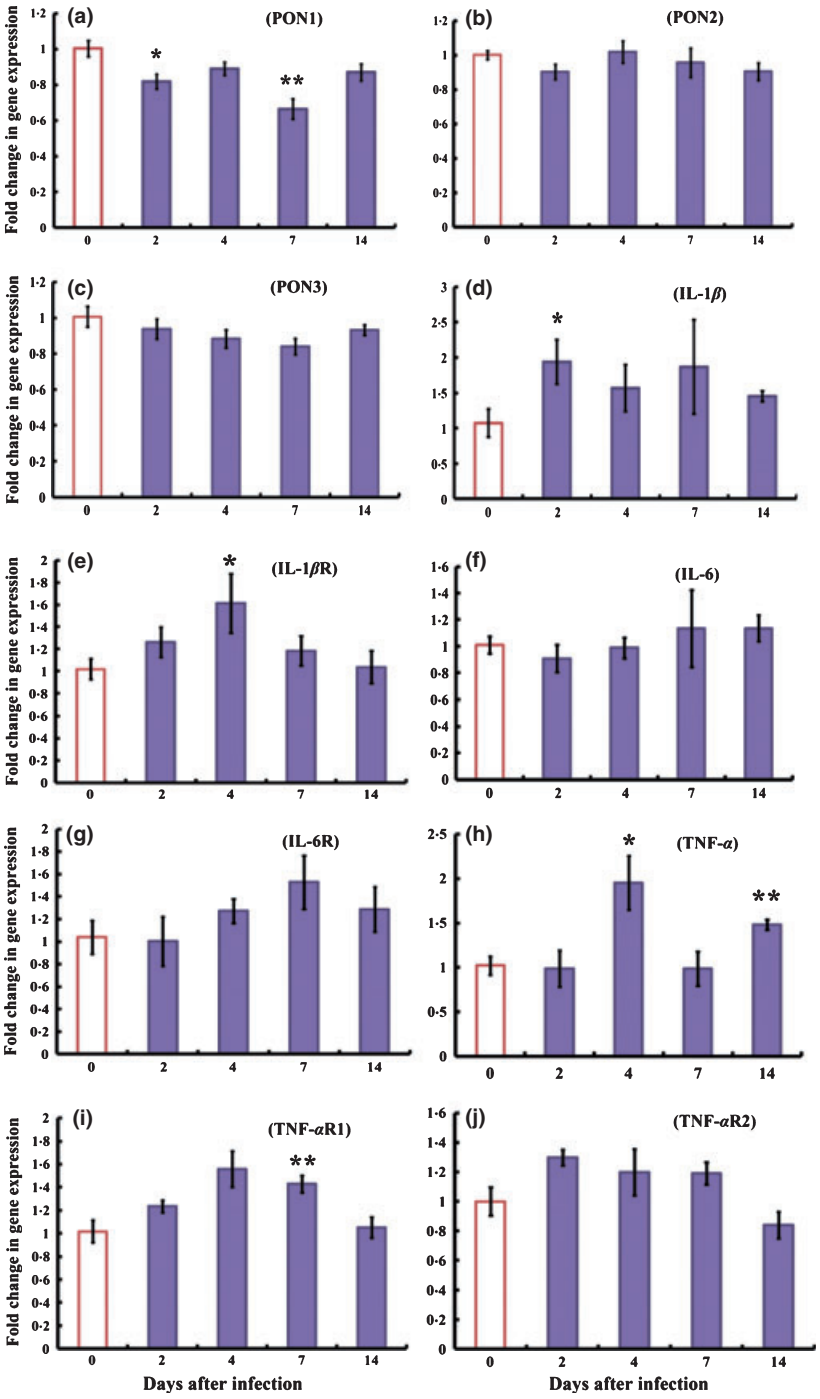

Figure 4 mRNA expression of hepatic PON (PON1, PON2 and PON3) genes (a-c) and pro-inflammatory cytokines and their receptors (IL-1β, IL-βR, IL-6, IL-6R, TNF-α, TNFR1 and TNFR2) (d-j). Total RNA was prepared from hepatic tissues of rats infected with *N. brasiliensis* fed standard diet on days 0, 2, 4, 7, and 14 p.i. The expression levels were evaluated by real-time PCR. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, compared with control values. Bars represent means ± SEM. (n = 4–5).

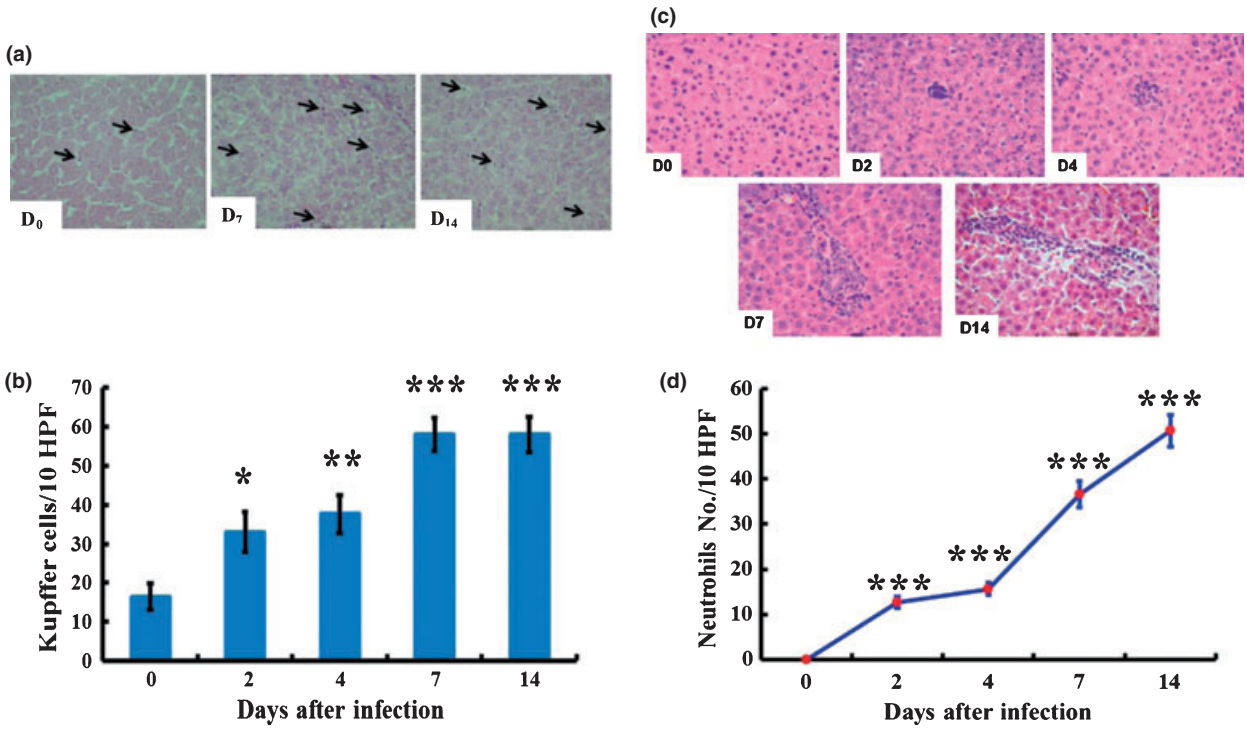

Figure 5 Kupffer cell and neutrophils response following infection with *N. brasiliensis*. Methyl green pyronin-stained sections of liver tissues and *N. brasiliensis*-infected rat (days 0, 7, and 14 p.i.) fed standard diet (a). Arrows indicate Kupffer cells. Number of Kupffer cells on days 0, 2, 4, 7 and 14 p.i with *N. brasiliensis* (b). Haematoxylin and eosin-stained sections of liver tissues of *N. brasiliensis*-infected rat (days 0, 2, 4, 7, and 14 p.i.) fed a standard diet (c). Numbers of neutrophils on days 0, 2, 4, 7 and 14 p.i with *N. brasiliensis* (d). $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ and $^{***}P < 0{\cdot}001$, compared with control value. Bars represent means ± SEM ($n = 5$). HPF means high power field.

Works by the authors Farid *et al.*[7] have confirmed that infection by the gastrointestinal nematode *N. brasiliensis* significantly reduces serum PON1 activity, thus increasing the sensitivity of rats to organophosphate toxicity [24]. Then we, in this study, went further to investigate the relation between serum PON1 level and its hepatic synthesis. We also examined the effects of reduced serum PON1 activity and associated changes during *N. brasiliensis* infection on lipid parameters.

We demonstrated that paraoxonase activity decreases significantly following *N. brasiliensis* infection on days 2 and 7 p.i. Arylesterase activity of PON1 also decreases significantly following infection with *N. brasiliensis* from day 2 until day 14 p.i. These results are consistent with our previous data showing a significant PON1 reduction during *N. brasiliensis* infection [7].

No previous studies have described the regulation of hepatic expression (synthesis, secretion and degradation) of PON1 during *N. brasiliensis* infection in rats. However, this study provides evidence that the reduced serum PON1 activity during *N. brasiliensis* infection in rats is closely correlated with hepatic PON1 mRNA expression (Fig. 4a). Importantly, the degree of PON1 mRNA down-regulation is proportional to the reduction in serum PON1 activity. This observation is supported by the results of the previous studies showing that serum PON1 activity is closely correlated with hepatic PON1 mRNA levels [25–27].

The mechanism by which hepatic PON1 mRNA is down-regulated during *N. brasiliensis* infection in rats is mostly induced by various pro-inflammatory cytokines associated with *N. brasiliensis* infection. This is supported by our finding of increased Kupffer cell numbers during *N. brasiliensis* infection. This observation suggests that cytokines are produced in response to *N. brasiliensis* infection by non-parenchymal liver cells, particularly intrahepatic macrophages (Kupffer cells) or extrahepatic macrophages, the number of neutrophils increases in hepatic tissues. This notion is supported by the up-regulation of hepatic IL-1β (day 2 p.i.), IL-1βR (day 4 p.i.), TNF-α (days 4 and 7 p.i.) and TNFR1 (day 7 p.i.) mRNA expression. However, the absence of increased IL-6 and IL-6R mRNA expression suggests a minimal role for IL-6 in hepatic PON1 down-regulation. These results are consistent with our previous results

showing increased serum levels of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) on day 9 p.i. [7], providing evidence that hepatic PON1 mRNA is down-regulated during *N. brasiliensis* infection in response to inflammatory conditions either in hepatic tissue or induced during larval migration. In addition, Kumon *et al.* [28] showed that hepatic PON1 is down-regulated following treatment TNF-α and IL-1. The role of pro-inflammatory cytokines in down-regulation of PON1 mRNA is thus primarily mediated by nuclear factor-κB (NF-κB) [29].

On the other hand, the mechanisms underlying the increased numbers of Kupffer cells following *N. brasiliensis* infection are unknown at present; however, it is known that Kupffer cells constitute the first macrophage population of the body to come into contact with microbial debris derived from the gastrointestinal tract and transported to the liver via the portal vein [30]. Moreover, *N. brasiliensis* infection is associated with intestinal inflammation [31], which is known to significantly increase the numbers of Kupffer cells [32,33]. This increase in Kupffer cell numbers during hepatic inflammatory conditions be the result of Kupffer cell recruitment from bone marrow, in addition to resident Kupffer cell proliferation in the liver [34]. The generation of this inflammatory response in the hepatic tissue leads to recruitment of inflammatory cells such as neutrophils [35], which may explain our findings of increased the number of neutrophils during *N. brasiliensis* infection. These inflammatory mediators then activate neutrophils in the hepatic microvasculature (sinusoids and post-sinusoidal venules) leading to a variety of events culminating in hepatocellular dysfunction [35].

High-fat diet-fed rats infected with *N. brasiliensis* showed significant increases in triglyceride levels on day 7 p.i., compared with days 0, 2, 4 and 14 p.i. This supports the previous results of Ovington [36], who found a significant increase in plasma triglyceride levels on day 9 following infection of rats with *N. brasiliensis*. The reason for this increase in triglyceride levels may be the increased levels of TNF-α during infection with *N. brasiliensis*, which peaked on day 9 p.i. [7]. TNF-α induces hypertrigylceridaemia by impairing its clearance from circulation as a result of loss of lipoprotein lipase activity, an enzyme responsible for catalysing triglyceride hydrolysis and clearing triglyceride from the blood [37], and by increasing hepatic lipogenesis [38,39]. Moreover, TNF-α is negatively correlated with HDL-C [40], and this may explain our finding of decreased HDL-C levels after infection with *N. brasiliensis*. In rodents, TNF-α and IL-1 induce increased serum cholesterol (total and LDL-C) via increased hepatic hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol synthesis and secretion, which leads to an increase in hepatic cholesterol synthesis, thus facilitating hepatic lipoprotein secretion, thereby contributing to host defence [41–44]. This may explain our results of increasing total cholesterol and LDL/VLDL levels at the same time as decreasing HDL-C, which may be caused by increased levels of TNF-α and IL-1 during *N. brasiliensis* [7].

Interestingly, we observed a significant increase in Ox-LDL levels on days 2 and 7 p.i. in high-fat diet fed rats in parallel with paraoxonase reduction during *N. brasiliensis* infection in standard-diet fed rats. The reason for LDL oxidation may be decreased HDL protective effects as a result of reduced PON1 activity [22,45] during *N. brasiliensis* infection. However, it is also possible that the increased levels of oxidative stress associated with *N. brasiliensis* infection in rats [46] contribute to lipid oxidation, which is manifested by increased levels of TBARS (Fig. 2b). The increased TBARS levels in our experimental model are consistent with the results of the study by Aviram *et al.* [22], who noted high levels of TBARS in the presence of PON-low HDL, indicating the important protective effects of HDL-associated PON against oxidation.

It is worth noting that the characteristic disturbances in PON1 activity and lipid metabolism manifested by low PON1 activity, increased serum total cholesterol, LDL and triglyceride concentrations, as well as decreased HDL-C, were triggered by nematode infection and the associated inflammation as a host response to infection and tissue destruction. When these compensatory responses are not able to repair injury, they can become harmful, and lipid changes become chronic as a result of either repeated or overwhelming stimuli, and this enhances the formation of atherosclerotic lesions [47]. This is particularly alarming in the light of the fact that the prevalence of intestinal worms in human populations remains virtually unchanged [9], despite the 'westernization' of diet and lifestyle among populations in developing countries, which may lead to an increased prevalence of atherosclerosis-related diseases [48]. Therefore, the results of this study may support the infectious theory of atherosclerosis [49].

We recognize some potential limitations of this study. First, the duration of this experimental model was short with regard to the chronic nature of atherosclerosis. Importantly, PON1 activity was significantly lower and indices of oxidative stress significantly higher in nematode-infected rats in comparison with the control groups, suggesting that the observed effect would be deleterious if continued for a long duration. Therefore, further studies using long lasting intestinal nematode infections would be helpful to resolve this issue. Second, rat is not very appropriate model for the study of atherosclerosis. However, there are a number of rat strains carrying mutations that lead to obesity, insulin resistance, hyperinsulinaemia, and hypertriglyceridsaemia [50]. One of these models can be used to provide more insight into our work.

In summary, this study indicates that the reduced PON1 activity during *N. brasiliensis* infection and associated disturbances in lipid metabolism might have a role in the development of atherosclerosis. Therefore, further investigation into

the effects of chronic nematode infections on the immunopathogenesis of atherosclerosis is of particular importance.

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