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Short communication

Acute phase protein and antioxidant responses in dogs with experimental acute monocytic ehrlichiosis treated with rifampicin

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ABSTRACT

There is currently lack of information on the changes of acute phase proteins (APP) and antioxidant markers and their clinical relevance as treatment response indicators in canine monocytic ehrlichiosis (CME). The objective of this study was to investigate the patterns of C-reactive protein (CRP), haptoglobin (Hp), ferritin and paraoxonase-1 (PON-1) during treatment of dogs with acute CME with rifampicin. Blood serum samples from ten Beagle dogs with experimental acute CME were retrospectively examined. Five dogs (Group A) were treated with rifampicin (10 mg/Kg/24 h), per os, for 3 weeks and 5 dogs (Group B) received no treatment (infected controls). Two Beagle dogs served as uninfected controls. Blood serum samples were serially examined prior to *Ehrlichia canis* inoculation and on post-inoculation days 14, 21, 28, 35 and 42. Significant changes of CRP, Hp, ferritin and PON-1 values were found in the majority of infected dogs. However, their concentrations did not differ between the two groups during the treatment observation period. The results of this study indicate that although several APP and PON-1 tend to significantly change in the majority of dogs with acute CME, they were of limited clinical relevance as treatment response indicators in this experimental setting.

1. Introduction

Ehrlichia canis is the major cause of canine monocytic ehrlichiosis (CME) worldwide. The clinical course of the experimental infection can be divided into acute, subclinical and chronic phases, although this distinction is not straightforward in the naturally-occurring infection (Neer et al., 2002).

Acute phase proteins (APP) constitute a component of the innate immune response of the host shortly after tissue injury, most commonly of infectious, immunologic, neoplastic or traumatic nature (Ceron et al., 2005). Accumulating evidence suggests that these non-invasive markers may be helpful in determining disease severity, prognosis and response to treatment, on a disease-specific basis (Ceron et al., 2005). In a previous study with dogs naturally infected with *E. canis*, C-reactive protein (CRP), haptoglobin (Hp) and serum amyloid A (SAA) concentrations on admission were useful indicators of the severity of the disease, but were unable to predict survival (Mylonakis et al., 2011). In experimental acute CME, CRP, SAA, Hp, ceruloplasmin, a1-acid glycoprotein and transferrin have all been shown to increase shortly after inoculation and subsequently decline to pre-inoculation levels even without treatment (Rikihisa et al., 1994; Shimada et al., 2002; M unhoz et al., 2012; Rudoler et al., 2015). Ferritin is a ubiquitous iron storage protein thought to be a positive APP and presumably a marker of oxidative stress (Martinez-Subiela

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et al., 2014). Paraoxonase-1 (PON-1) is an important enzyme involved in lipid metabolism and oxidation which is down-regulated during oxidative stress; it is also reported as a negative APP in dogs and other species (Martinez-Subiela et al., 2014; Rudoler et al., 2015). To the authors' knowledge, the changes of selected APP or antioxidant markers in the response to treatment in dogs infected with *E. canis* has not been previously reported. Therefore, the objective of this study was to investigate the patterns of CRP, Hp, ferritin and PON-1 during treatment of dogs with experimental acute CME with rifampicin.

2. Materials and methods

2.1. Experimental dogs

Serum samples from ten Beagle dogs experimentally infected with *E. canis* and two healthy uninfected Beagles were serially examined retrospectively. All dogs had participated in a previous study conducted in the CAC-AUTh, that evaluated the efficacy of rifampicin in the treatment of experimental acute CME (Theodorou et al., 2013; study approved by the Research and Ethical Committee, School of Veterinary Medicine, Aristotle University of Thessaloniki, 458/23-6-2009). Five of the dogs were males and 7 females, with an age ranging from 5 to 70 months (median: 11 months). Ten dogs had been infected by either intravenous inoculation with 5 ml of heparinized *E. canis*-infected blood drawn from a reservoir Beagle artificially infected with the Israeli *E. canis* strain "611" ($n=9$), or with an intravenous inoculum of *E. canis* strain "611"-infected DH82 cell culture ($n=1$). Before the infection, all dogs were healthy, had no clinicopathological abnormalities, were seronegative to *E. canis, Babesia canis*, *Leishmania infantum* (indirect fluorescence antibody, IFA) and to *Dirofilaria immitis* (Snap 3Dx, IDEXX, USA) and were polymerase chain reaction (PCR) negative for *E. canis* DNA in blood, spleen, and bone marrow (BM) aspirates. In addition, cytological evaluation of buffy coat smears was negative for *Ehrlichia* spp., *Anaplasma* spp. morulae and *Hepatozoon canis* gamonts. All dogs enrolled in the present study had serial clinical examinations performed on days 30, 14, 8, 6, 4, and 2 before and on the day of *E. canis* inoculation (day 0); thereafter, clinical examination was performed every 2 days until day 42 post-inoculation (PID). By PID 21, all infected dogs became clinically ill experiencing palpable splenomegaly ($n=9$), fever ($n=9$), lymphadenomegaly ($n=5$), anorexia ($n=4$), depression ($n=2$) and mucosal petechiae or pallor ($n=1$). In addition, all dogs became thrombocytopenic (platelet counts on PID 21: median: 61,500/μl, range: 44,000–101,000/μl, reference interval: 200,000–500,000/μl), seroreacted to *E. canis* antigens (median reciprocal IFA titers on PID 21:200, range: 200–1600, cut-off value: ≥100) and were PCR-positive in at least one tissue (i.e., blood, BM, or spleen) (Theodorou et al., 2013). The infected dogs were randomly allocated into two groups. Group A ($n=5$) included dogs that were treated with rifampicin (Rifantin, IFET, Greece) at 10 mg/Kg/24 h, per os, for 3 weeks (from PID 21 through PID 42). Group B ($n=5$) included dogs that received no treatment (infected controls). Two Beagle dogs served as uninfected controls and remained healthy, free of clinicopathological abnormalities, seronegative and PCR negative for *E. canis* throughout the study period. In the original study by Theodorou et al. (2013), it was shown that although a rifampicin-mediated clinical improvement could not be substantiated, rifampicin-treated dogs experienced resolution of thrombocytopenia significantly earlier and their median platelet counts were significantly higher compared to the untreated infected dogs on post-inoculation day 42. For the purpose of the present study, blood serum samples were available from day 0 (prior to inoculation) ($n=12$ dogs), and from PID 14 ($n=12$), 21 ($n=9$; i.e., 5 Group A and 4 Group B dogs), 28 ($n=5$; i.e., Group A dogs), 35 ($n=10$; i.e., Group A and B dogs) and 42 ($n=12$). Until analyzed, the serum samples were stored at −20 °C, for a 36 months period.

2.2. CRP, Hp, ferritin and PON-1 measurements

The collected serum samples were frozen-packaged and delivered to the Laboratory of Clinical Pathology, Faculty of Veterinary Medicine, University of Murcia, Spain, for CRP, Hp, ferritin and PON-1 measurement.

The methodology applied for the CRP and Hp measurements has been previously described (Mylonakis et al., 2011). Briefly, CRP

Table 1
Frequency of the changes and median concentrations of CRP, Hp, Ferritin and PON-1 in 10 dogs with experimental CME that were treated (Group A, $n=5$) or not (Group B, $n=5$) with rifampicin.

Variable (reference interval)	**Time** (**days post-inoculation**)					
	0	**14**	**21**[c]	**28**	**35**	**42**
Group A	**Increased or decreased concentrations/dogs tested [median, range]**					
CRP	0/5	5/5	5/5	0/5	0/5	0/5
(<20 mg/L)	[5, 5–7.7]	[118,75.8–247][a]	[51,47.1–132][a]	[5,5–17]	[5,5–5]	[5,5–5][b]
Hp	1/5	4/5	3/5	1/5	0/5	0/5
(<3 mg/L)	[1.65, 0.94–3.27]	[3.42, 2.52–4.49][a]	[3.43, 1.06–3.96][a]	[1.94, 0.42–3.32]	[1.34, 0.33–2.9]	[1.31, 0.38–2.77][b]
Ferritin	0/5	0/5	1/5	0/5	0/5	0/5
(60–190 μg/L)	[85.5, 64.7–122.1]	[89.5, 80.1–116.7]	[135.9, 105.6–267.9][a]	[102.9, 68–133.8]	[77.8, 46.6–118.4]	[84.8, 51.6–165.1][b]
PON-1	1/5	4/5	2/5	1/5	3/5	5/5
(2.5–3.1 IU/ml)	[2.72, 2.55–3.37]	[2.35, 2.18–2.59][a]	[2.96, 2.71–3.52]	[2.78, 2.60–4.02]	[3.19, 2.86–3.77]	[3.53, 3.45–4.13][b]
Group B	**Increased or decreased concentrations/dogs tested [median, range]**					
CRP	1/5	5/5	3/4	ND	1/5	0/5
	[9.5, 5–20.4]	[60.1, 42.9–88.5][a]	[34.3, 5–84.2]		[5,5–58.3]	[5,5–5]
Hp	1/5	1/5	1/4	ND	1/5	1/5
	[0.46, 0.41–3.32]	[1,0.47–4.86]	[1.95, 1.18–4.41]		[1.09, 0.87–3.33]	[0.74, 0.49–3.26]
Ferritin	0/5	0/5	0/4	ND	0/5	0/5
	[83.5, 31.2–107.3]	[113.3, 58.7–143.3]	[81.3, 62.3–143.7]		[72.7, 70.7–109.7]	[77.1, 72.4–99.6]
PON-1	4/5 [3 (1)]	3/5	3/4 [1 (1)]	ND	3/5	5/5
	[2.47, 2,13–3.19]	[2.40, 2.13–2.89]	[3.02, 1.92–3,48]		[3.21, 3.03–3.50]	[3.68, 3.35–3.88]

CME: Canine monocytic ehrlichiosis; CRP: C-reactive protein; Hp: Haptoglobin; PON-1: Paraoxonase-1; ND: not done. (↓): Decreased concentrations of PON-1.
[a] Significant changes ($P<0.05$) of the median concentrations between 14 or 21 days post-inoculation compared to time 0.
[b] Significant changes ($P<0.05$) of the median concentrations in the overall trend from time 21 days post-inoculation to 42 days post-inoculation.
[c] Start of rifampicin treatment in Group A dogs.