

### Short communication

# ACUTE-PHASE RESPONSE AND THE EFFECT OF PHYTOPREPARATION FEVERFEW (TANACETUM PARTHENIUM) IN DOGS WITH EXPERIMENTAL PSEUDOMONAS AERUGINOSA SKIN INFECTION

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#### Summary

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Acute-phase response is most critical for the attenuation of the strong inflammatory response induced by bacterial opportunist *Pseudomonas aeruginosa*. The aim of the present study was to evaluate the acute-phase response in experimental Pseudomonas aeruginosa skin infection, by measuring changes in fibrinogen concentration (a positive acute-phase protein, APP) and activity of arylesterase (ARE a negative APP). We also aimed to evaluate the effect of therapy with phytopreparation Feverfew, containing the active component parthenolide, which has anti-inflammatory properties. Fifteen male mongrel dogs at 2-5 years of age were divided into three groups: group 0 (n=5) with infection induced by subcutaneous injection of bacterial *Pseudomonas aeruginosa* culture  $1 \times 10^8$  CFU/mL; group I (n=5) – infected and treated with Feverfew (standardised extract, active principle parthenolide 0.7% - Nature's Way, USA), by application of 1 capsule at 12-hour intervals. The per os treatment began on post infection hour 4 and continued for 6 days. Dogs from group C (n=5) were controls. Plasma fibrinogen and serum ARE activity were assayed before infection and on 4<sup>th</sup>, 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> hour and on 7<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> day after infection. The results suggested that fibrinogen levels in dogs from group 0 increased on p.i. hour 24 vs baseline, attained a peak on hour 48 (P<0.001), and persisted high on hour 72. Infected dogs treated with Feverfew (group I) exhibited a similar time course of changes in fibrinogen levels, but the numeric values were lower compared to those of group 0 (P<0.05). ARE activity in experimental animals did not change significantly and was similar to control values.

Key words: acute-phase response, arylesterase, Feverfew, fibrinogen, skin *Pseudomonas* aeruginosa infection

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Pseudomonas aeruginosa is an opportunistic pathogen that induces a strong inflammatory response, accompanied by considerable systemic effects (Epelman et al., 2004; Kumar et al., 2011). These changes represent an immediate set of reactions counteracting the challenge, aimed at minimisation of tissue damage, initiation and promotion of repair processes as well as the isolation and neutralisation of pathogen and the prevention of further pathogen entry. Acute phase response (APP) is the one of endogenous mechanisms for control of systemic inflammatory response (Bode et al., 2012). Many acute phase proteins (APPs) synthesised in the liver are multifunctional, playing different functions. For example, fibrinogen is not only important for clot formation (Stassen et al., 2004) but also for the recruitment of leukocytes and increasing the local concentration of factors for defense at the site of tissue injury (Farrell, 2004). Arylesterase (ARE) has a direct antibacterial effect on P. aeruginisa growth (Ceron et al., 2014). The multifactorial nature of ARE includes characteristics of a negative APP (Rossi et al., 2013). Tang et al. (2012) and Li et al. (2013) comment on the prognostic value of serum ARE activity in patients with sepsis as an antioxidant bio-scavenger, responsible for hydrolysing lipid peroxides and playing a major role in the antioxidant system.

Further investigations are required to achieve detailed knowledge of the regulation and function of the different APPs and their mutual interrelationship. The effective application of APPs in the clinical practice requires assaying not only a single APP, but a precise combination of positive and negative APPs, utilisation of biomarkers (Gruys, 2002) for easier differentiation of healthy animals from infected ones, as well for evaluation of therapy efficacy (Faure *et al.*, 2014). These proteins may be of interest as target structures for novel approaches to cope with inflammation. The optimisation of strategies for control of *Pseudomonas aeruginosa* requires use of medications that possess good anti-inflammatory therapeutic potential (George *et al.*, 2012; Vitiello *et al.*, 2012). The aim of such a therapy is to limit the intensity of inflammatory response. Parthenolide is a natural product derived from the medicinal plant Feverfew with anti-inflammatory properties (George *et al.*, 2012).

The aim of the present study was to evaluate the acute phase response in experimental *Pseudomonas aeruginosa* skin infection in dogs, by measuring changes in fibrinogen concentration (a positive APP) and activity of arylesterase (ARE – a negative APP). We also aimed to evaluate the effect of therapy with phytopreparation Feverfew, applied in the early stage of skin infection.

Fifteen male mongrel dogs at 2–5 years of age, were housed at room temperature: 15–21°C, humidity 50–60%, regulated light-dark regimen (12h/12h) and were fed a standard maintenance diet (Canil Social Gouomarc H, Brazil) with free access to water. Experiments were performed with strict compliance with animal ethics standards (protocol 16/2010 of the Ethical Committee of National Veterinary Service; permit No. 37).

Animals were divided into three groups. In dogs from group 0 (n=5), infection was induced by subcutaneous injection of *Pseudomonas aeruginosa* bacterial culture in stationary phase with density corresponding to  $1 \times 10^8$  CFU/mL prepared nephelometrically according to the Mac-Farland standard. Dogs from group I (n=5) were treated with Feverfew (standardised extract, active principle parthe-

nolide 0.7%, Nature's Way, USA), by application of 1 capsule at 12-hour intervals. The per os treatment began on post infection hour 4 and continued for 6 days. Dogs from group C (n=5) were controls.

Blood samples were obtained from v. cephalica antebrachii by means of Venflon cannulae (Vygon GmbH & Co., Germany) prior to the infection (hour 0) and on post infection (p.i.) hours 4, 24, 48, 72 and days 7, 10 and 14. All blood samples were collected in the morning before feeding (8.00-8.30 AM) to eliminate circadian influences. Samples were allowed to clot for two hours at room temperature before centrifugation for 15 min at 1000×g. Sera were removed, assaved immediately or aliquoted and stored at -80 °C. Plasma fibrinogen concentration was analysed by the method of Podmore (1959) and serum arylesterase activity was assayed by the method of Lorentz et al. (1979). Results are presented as mean  $\pm$  SD. Data were submitted to oneway ANOVA test (Graph Pad InStat3). Differences were considered statistically significant at the P<0.05 level.

The results from the study are presented in Table 1. Fibrinogen levels in dogs from group 0 increased on p.i. hour 24 vs baseline (p < 0.01), attained a peak on hour 48 (P<0.001), and persisted high till hour 72 (P<0.001). At that time, they were statistically higher than respective values in control dogs (P<0.001). The reduction in fibrinogen concentrations in subsequent periods was statistically significant both vs group C (P<0.01) and vs levels on hour 72 (P<0.05 by p.i. day 10; P<0.01 by p.i. day 14) of Pseudomonas aeruginosa infection. Infected dogs treated with Feverfew (group I) exhibited a similar time course of changes in fibrinogen concentrations, but the numeric values were lower compared to those of group 0 (P < 0.05). The peak observed by

**Table 1.** Changes in fibrinogen (g/L) and arylesterase (U/L) concentrations in control dogs (Group C, n=5), dogs with experimental *Pseudomonas aeruginosa* infection (Group 0, n=5) and infected dogs treated with the phytopreparation Feverfew (Group I, n=5)

	Time periods								
	0 h	4 h	24 h	48 h	72 h	7 d	10 d	14 d	
	Fibrinogen (g/L)								
Group C	1.5±0.2	$1.6\pm0.47$	$1.6\pm 0.37$	$1.7\pm0.50$	1.7±0.44	1.6±0.28	1.6±0.40	$1.6\pm 0.35$	
Group 0	2.0±0.55	4.4±0.66 <i>a3</i>	5.6±1.57 <i>a3;**</i>	6.2±1.32 <i>a3;***</i>	5.7±1.98 <i>a3;</i> ***	4.0±1.68 a2	3.6±1.56 <i>a2;</i> #	3.1±1.22 <i>a2; v; ##</i>	
Group I	$1.4{\pm}0.08$	1.9±0.86	3.3±1.16	4.9±1.36	4.2±0.44	1.7±0.07	1.3±0.07	$1.3 \pm 0.07$	
		<i>b3</i>	<i>b1;*</i> *	a1;***; +++;§	<i>a1;b1;</i> ***;+++	<i>b2 §;vvv;</i> ###	b3; §§;vvv; ###	b2; §§;vvv; ###	
Arylesterase activity (U/L)									
Group C	62.2±6.47	$62.3 \pm 7.28$	$61.8 \pm 10.5$	58.6±20.5	55.7±6.16	$61.0{\pm}16.7$	$61.6 \pm 5.90$	60.3±4.99	
Group 0	61.6±2.70	53.9±6.26	64.1±8.44	64.4±9.58	55.7±17.4	65.4±3.20	68.6±4.79	$60.4{\pm}1.48$	
Group I	55.4±11.0	53.9±21.4	74.2±21.8	75.6±22.2	51.9±12.2	65.8±30.1	58.1±19.4	52.4±22.2	

*Legend*: Within-group statistical significance: P<0.05; \*\*P<0.01; \*\*\*P<0.001 vs 0 h; +P<0.05; ++P<0.01; +++P<0.001 vs 4 h; P<0.05; P<0.01; P<0.01; P<0.05; P<0.01; P<0.05; P<0.01; P<0.05; P<0.01; P<0.05; P<0.01; P<0.01; P<0.01; P<0.05; P<0.01; P<0.01; P<0.01; P<0.01; P<0.05; P<0.01; P<0.01;

p.i. hour 48 was  $4.9\pm1.36$  g/L, unlike the peak in group 0:  $6.2\pm1.32$  g/L. Within p.i. hours 48 and 72, the differences vs group C were statistically significant (P<0.01), and afterwards, average fibrinogen concentrations attained  $1.7\pm0.07$  g/L – by the 7<sup>th</sup> day (P<0.001 vs 72 h), and  $1.3\pm0.07$  g/L on days 10 and 14 (P<0.001 vs 72 h). ARE activity in experimental animals did not change significantly and was similar to control values.

In our experimental model of P. aeruginosa skin infection we have analysed the acute-phase response in dogs by measuring the changes in two APPs fibrinogen being a positive APP, and ARE being a negative one. Results show that fibrinogen concentrations in infected animals mark a 2-3 fold increase in the period within 24 to 72 hours post infection. This finding is consistent with results of other researchers (Georgieva et al., 2013; Zapryanova et al., 2013), who have studied Staphylococcus aureus experimental infections. This suggests that fibrinogen is a non-specific marker of infection, but it can also be an indicative parameter in P. aeruginosa skin infection in dogs. The assav method is low-cost and easy to perform, which makes it applicable for this animal species. The coagulation system is closely linked to inflammation predominately through the innate immune response (Hanington & Zhang, 2011). The attempt to correct the balance between non-specific immune mechanisms during P. aeruginosa infection, by treating dogs from group I with Feverfew demonstrates that this medication did not change the dynamics, but only the magnitude of increase of fibrinogen levels. This is probably linked to the inhibitory effect of phytopreparation Feverfew on platelet aggregation and secretion (Biggs et al., 1992). ARE activity did not show any statistically

significant differences. In contrast, Tvarijonaviciute et al. (2012) have found 1.2 fold decrease of ARE after experimental LPS administration to dogs. Rossi et al. (2013) have also documented decrease of ARE during inflammation. Although, most of the studies show decreased ARE activity, some studies suggest no difference in enzyme activity (Erdem et al., 2010; Oran et al., 2014). Toker et al. (2009) reported that serum ARE activities were significantly higher in patients with psoriasis than in controls. These conflicting results may be explained by the effects of several factors. ARE activity may depend on species, sex, age (Tvarijonaviciute et al., 2012) and nutrition (de la Iglesia et al., 2014). Our experimental model aimed to minimise the influence of such factors. Genetic differences may also be a reasonable explanation for this discrepancy (Oran et al., 2014). The methods of ARE assay may also influence the results (Tvarijonaviciute et al., 2012). However, further research is needed to elucidate the exact mechanism leading to changes in ARE activity in skin infection induced by P. aeruginosa in dogs. In infected dogs treated with Feverfew we found no changes in ARE activity, although this phytopreparation has been found to possess analgesic, anti-inflammatory and antipyretic properties similar to those of aspirin. Salman et al. (2011) have found that aspirin is a PON 1 activator.

In conclusion, blood fibrinogen levels in dogs with experimental skin *P. aeruginosa* infection increased 2–3 times between p.i. hours 24 and 72 vs non-infected dogs. This provides sufficient information about the progression of skin *P. aeruginosa* infection in dogs. The applied phytopreparation Feverfew did not alter the course, but only the magnitude of increase in this positive acute phase protein. AryAcute-phase response and the effect of phytopreparation Feverfew (Tanacetum parthenium) in dogs...

lesterase activity in dogs with experimental skin infection did not change. The activity of the enzyme has neither changed in infected dogs treated with Feverfew.

#### REFERENCES

- Biggs, M. J., E. S. Johnson, N. P. Persaud & D. M. Ratcliffe, 1992. Platelet aggregation in patients using feverfew for migraine. *Lancet*, 2, 776.
- Bode, J., U. Albrecht, D. Haussinger, P. Heinrich & F. Schaper, 2012. Hepatic acute phase proteins – regulation by IL-6 and IL-1-type cytokines involving STAT3 and its crosstalk with NF-kB-dependent signaling. *European Journal of Cell Biol*ogy, **91**, 496–505.
- Ceron, J., F. Tecles & A. Tvarijonaviciute, 2014. Serum paraoxonase 1 (PON1) measurement: An update. *BMC Veterinary Research*, **10**, 1–11.
- de la Iglesia, R., M. L. Mansego, F. J. Sanchez-Muniz, M. A. Zulet & A. A. Martinez, 2014. Arylesterase activity is associated with antioxidant intake and paraoxonase-1 (PON 1) gene methylation in metabolic syndrome patients following an energy restricted diet. *EXCLI Journal*, **13**, 416–426.
- Epelman, S., D. Stack & C. Bell, 2004. Different domains of *Pseudomonas aeruginosa* exoenzyme S activate distinct TLRs. *Journal of Immunology*, **173**, 2031–2040.
- Erdem, F. H., S. Karatay, K. Yildirim & K. Kiziltunc, 2010. Evaluation of serum paraoxonase and arylesterase activities in ankylosing spondylitis patients. *Clinics*, 65, 175–179.
- Farrell, D. H., 2004. Pathophysiologic roles of the fibrinogen gamma chain. *Current Opinion in Hematology*, **11**, 151–155.
- Faure, E., J. B. Mear, K. Faure, S. Normand, A. Couturier-Maillard, T. Grandjean, V. Balloy, B. Ryffel, R. Desssein, M. Chignard, C. Uyttenhove, B. Guery, P. Gosset, M. Chamaillard & E. Kipnis, 2014. *Pseu-*

domonas aeruginosa type 3 secretion system dampens host defense by exploiting the NLRC4-couple inflammasome. American Journal of Respiratory and Clinical Care Medicine, **189**, 7, DOI:10.1164/ rccm.201307-13580C.

- George, V. C., D. R. N. Kumar, P. K. Suresh & R. A. Kumar, 2012. A review on the therapeutic potential of parthenolide: A sesquiterpene lactone. *International Research Journal of Pharmacology*, 3, 69–73.
- Georgieva, T. M., D. Zapryanova, I. Nikiforov & S. A. Denev, 2013. Plasma C-reactive protein concentration in dogs with experimentally-induced *Staphylococcus aureus* infection. *Revue de Médicine Vétérinaire*, 164, 156–161.
- Gruys, E., 2002. Acute phase proteins in bovine medicine. In: AVMA Convention Notes. Proceedings of the American Veterinary Medical Association. Nashville, pp. 317–321.
- Hanington, P. C. & S. Zhang, 2011. The primary role of fibrinogen-related proteins in invertebrates is defense, not coagulation, *Journal of Innate Immunity*, 3, 17–27.
- Kumar, H., T. Kawai & S. Akira, 2011. Pathogen recognition by the innate immune system. *International Reviews of Immunolo*gy, **30**, 16–34.
- Li, Y., R. Zhai, H. Li, X. Mei & G. Qui, 2013. Prognostic value of serum paraoxonase and arylesterase activity in patients with sepsis. *Journal of International Medical Research*. DOI: 10.1177/030006051348 3412.
- Lorentz, K., B. Flatter & E. Augustin, 1979. Arylesterase in serum: Elaboration and clinical application of a fixed-incubation method. *Clinical Chemistry*, 25, 1714– 1720.
- Oran, M., F. Tulubas, R. Mete, M. Aydin, H. G. Sarikaya & A. Gurel, 2014. Evaluation of paraoxonase and arylesterase activities in patients with irritable bowel syndrome. *Journal of the Pakistan Medical Association*, 64, 820–822.

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- Podmore, D., 1959. Rapid turbidimetric methods for the determination of plasma fibrinogen. *Clinica Chimica Acta*, **4**, 242– 245.
- Rossi, G., A. Giordano, F. Pezzia, M. Kjelgaard-Hansen & S. Paltrinieri, 2013. Serum paraoxonase activity (PON1) in dogs: Preanalytical and analytical factors and correlation with C-reactive protein and alpha-2-globulin. *Veterinary Clinical Pathology*, **42**, 329–341.
- Salman, M., C. Malleda, N. A. Suneel, I. A. Qureshi, E. A. Frank & C. J. M. D'Souza, 2011. Homology modelling of human serum paraoxonase 1 and its molecular interaction studies with aspirin and cefazolin. *Bioformation*, 7, 59–63.
- Stassen, J. M., J. Arnout & H. Deckmyn, 2004. The hemostatic system. *Current Medical Chemistry*, 11, 2245–2260.
- Tang, W. H. W., J. Hartiana, Y. Wu, A. F. R. Stewart, J. Erdmann, S. Kathiresan, R. Roberts, R. McPherson, H. Allayee & S. E. Hazen, 2012. Clinical and genetic association of serum paraoxonase and arylesterase activities with cardiovascular risk. *Arteriosclerosis, Thtombosis and Vascular Biology*, **32**, 2803–2812.
- Toker, A., M. Kadi, A. K. Yildirim, H. Askoy & F. Akcay, 2009. Serum lipid profile paraoxonase and arylesterase activities in psoriasis. *Cell Biochemistry and Function*, 27, 176–180.

- Tvarijonaviciute, A., M. Kocaturk, M. Cansev, F. Tecles, J. J. Ceron & Z. Yilmaz, 2012. Serum butyrylcholinesterase and paraoxonase 1 in a canine model of endotoxemia: Effects of choline administration. *Research in Veterinary Science*, **93**, 668–674.
- Vitiello, M., M. Galdiero, E. Finamore, S. Galdiero & M. Galdiero, 2012. NF-kB as a potential therapeutic target in microbial diseases. *Molecular BioSystems*, 8, 1108–1120.
- Zapryanova, D., T. Mircheva & S. A. Denev, 2013. Plasma protein profiles and fibrinogen concentrations in dogs with experimentally induced *Staphylococcus aureus* infection. *Revue de Médicine Vétérinaire*, 164, 150–155.

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