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In vivo administration of acepromazine or promethazine to horse decreases the reactive oxygen species production response of subsequently isolated neutrophils to stimulation with phorbol myristate acetate

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The previous experiments have shown that some phenothiazines have antioxidant and anti-inflammatory properties *in vitro*. In this study the inhibition of the production of reactive oxygen species (ROS) by neutrophils was studied in two groups of horses, which received a dose of 0.1 mg/kg of either acepromazine or promethazine intravenously. Blood samples were collected before (T0) and 0.5, 1, 3 and 5 h after drug administration. The chemiluminescence (CML) response of neutrophils was measured *ex vivo* in the presence of luminol for a period of 10 min and the maximum CML value (peak value) recorded. There was a significant inhibition of the ROS production in the acepromazine treated group (49% inhibition) at 5 h after administration and in the promethazine group (24% inhibition) at 3 h after administration ($P < 0.05$ vs. T0). These findings are of therapeutic relevance in the use of phenothiazines in equine patients with inflammatory diseases where neutrophil activation and ROS production are implicated.

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INTRODUCTION

The phenothiazine (PHE), acepromazine (ACP), has been used for a long time for sedation, treatment of equine laminitis and as premedication before general anaesthesia. It exerts sedative, anti-arrhythmic and vasodilator properties, which are considered to explain the protective effect of this drug against perioperative mortality during general anaesthesia (Johnston *et al.*, 2002). In particular, the vasodilation seems to decrease the haemodynamic disturbances of α2-agonists, when both drugs are administered concomitantly and probably increases arterial oxygenation by maintaining cardiac output during total intravenous (i.v.) anaesthesia in normovolemic horses (Marntell *et al.*, 2005). In addition, ACP is still the molecule of second choice in the treatment of horses with acute laminitis (Slater *et al.*, 1995). Its vasodilatory effect can explain some of its beneficial therapeutic properties (Ingle-Fehr & Baxter, 1999), but other pharmacological properties, such antioxidant and anti-inflammatory effects may contribute also to the therapeutic result. For example, the excessive activity of equine polymorphonuclear neutrophils and the early release of myeloperoxidase (MPO) and reactive oxygen species (ROS) occurring during the prodromic stage of laminitis (Hurley *et al.*, 2006; Loftus *et al.*, 2007; Riggs *et al.*, 2007) are potential targets for therapeutic intervention.

A number of PHEs including thioridazine, trifluoperazine and chlorpromazine have very interesting immunomodulating activities, depending on the drug, the dose and the leucocyte model used. PHEs inhibit the superoxide anion ($O_2{\cdot}^-$) production of phorbol myristate acetate (PMA)-stimulated human neutrophils (Szuster-Ciesielska *et al.*, 2004), modulate degranulation (Elferink, 1979; Richter, 1990; Blackwood & Hessler, 1995) and result in the formation of immature neutrophils when used over longer periods in the treatment of schizophrenic patients (Delieu *et al.*, 2001). At high doses, they diminish the motility of T-lymphocytes as well as the production of TNF-α and IL-1β (Steele & Brahmi, 1988; Matthews *et al.*, 1995; Grabski *et al.*, 2001; Kowalski *et al.*, 2001; Kiku *et al.*, 2002).

In vivo experiments demonstrated that chlorpromazine decreases mortality and prevents typical biological effects of

lipopolysaccharide (LPS) such as the release of inflammatory cytokines like TNF-α and IL-1β in rodents (Boraschi *et al.*, 1991; Ghezzi *et al.*, 1996; Clancy *et al.*, 2000), dogs (Molnar *et al.*, 1992) and calves (Ohtsuka *et al.*, 1997). ACP inhibits the differentiation of monocytes into macrophages and diminishes monocyte TNF-α production (Serteyn *et al.*, 2001, 2002). In some conditions, these effects were compared with those of dexamethasone and the results generally advantage chlorpromazine, which is a more specific inhibitor of TNF-α production and increases the induction of IL-10, a 'protective' cytokine known to reduce LPS dependant inflammation (Gadina *et al.*, 1991; Mengozzi *et al.*, 1994). A review of the effects of phenothiazines, on the cytokine networks was presented by Pollmacher *et al.* (2000). Moreover, PHEs have shown to inhibit directly or indirectly protein kinase-C (PKC) (Aftab *et al.*, 1991; Chen *et al.*, 2001), phospholipase (Wightman *et al.*, 1981; Watanabe *et al.*, 1986; Jain *et al.*, 1991; Brufani *et al.*, 1992; Babu & Gowda, 1995), NADPH-oxidase (Jones *et al.*, 1982) and carboxylesterase (Radenovic & Kartelija, 2004). Finally, like tocopherol, ACP has an antioxidant activity, which is interesting in pathophysiological situations where there is excessive formation of ROS (Serteyn *et al.*, 2000). In humans, controversial results have been found regarding the influence of chlorpromazine on spontaneous cytokine production, as the doses of chlorpromazine that can be safely given to humans failed in some experiments to inhibit TNF-α and IL-1β release (Bleeker *et al.*, 1997). However, with doses close to those used in equine practice, ACP and promethazine (PTZ) decrease the production of ROS by activated neutrophils *in vitro* (Traykov *et al.*, 1997; Serteyn *et al.*, 1999).

According to Ghezzi *et al.* (1996), the inhibition of TNF-α production by chlorpromazine is likely to be related to these antioxidant properties. The free radical scavenging effect of PHEs, probably by linkage to their aromatic ring, has also been well described (Dalla Libera *et al.*, 1998; Hadjimitova *et al.*, 2002, 2004).

The aim of this study was to demonstrate that the *in vivo* administration of ACP and PTZ to horses has antioxidant properties by interacting with the capacity of equine neutrophils to react to a stimulation ex vivo. Both compounds were administered i.v. at the same dose of 0.1 mg/kg body weight to two groups of adult horses and neutrophils were isolated from the peripheral blood of these horses at different time points after treatment. Neutrophils were simulated *ex vivo* with PMA, a direct intracellular stimulator of PKC, which activates NADPH-oxidase, the main responsible enzyme for ROS production. Subsequently, the production of reactive oxidant species (ROS) was measured by chemiluminescence (CML).

MATERIALS AND METHODS

Animal selection and blood sampling

Six healthy adult warmblood horses (Group A: three mares – two geldings – one stallion in a body range of 412–589 kg) without medication for at least 1 week received 0.1 mg/kg ACP (Combisstress®, 20 mg/mL; KELA Laboratoria N.V., Hoogstraaten, Belgium) i.v. A second group of warmblood horses (Group B: three mares – three geldings in a body range of 453–602 kg) received 0.1 mg/kg PTZ (Phénergan®, 25 mg/mL; Rhône-Poulenc Rorer S.A., Brussels, Belgium). Blood samples (300 mL) were collected before (T0) and 0.5, 1, 3 and 5 h after the PHE injection. To avoid the potential effect of repeated venipuncture on neutrophil activity, the blood was collected through a catheter placed in the left jugular vein the day before the study. Blood samples were collected into citrated transfusion bags (Baxter transfusion pack®, PL146 – CPD; Baxter Healthcare Corporation, Deerfield, IL, USA), refrigerated at 4 °C and processed within 1 h.

Isolation of neutrophils

Neutrophils were isolated at room temperature (18–22 °C) by centrifugation (400 ***g***, for 35 min, at 20 °C) on a discontinuous Percoll density gradient (Polymorphprep™; Nycomed Pharma S.A., Diagnostics, Oslo, Norway) according to the method of Boyum (1976), modified by Pycock *et al.* (1987). The polymorphonuclear fraction was gently collected in the inferior band of the gradient and washed in two volumes of Hanks' balanced salt solution (137 mM NaCl, 5.4 mM KCl, 4.2 mM $NaHCO_3$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4; pH 7.4). The cells were re-suspended at 4 °C in a phosphate-buffered saline solution (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) and counted (Burker cell). The cell preparation contained ≥95% neutrophils with viability ≥95% as measured by Trypan blue exclusion test.

Measurement of the CML response of activated neutrophils

The ROS production was measured by CML according to the method of Easmon *et al.* (1980), adapted to equine neutrophils by Benbarek *et al.* (1996). Neutrophils (1.25×10^6 cells/assay) were diluted in 400 μL of Hanks' balanced salt solution with luminol added at the final concentration of 10^{-4} M. For each isolated cell suspension, two *in vitro* experiments were conducted. In the first experiment, basal CML response of 1.25×10^6 nonstimulated neutrophils was recorded to evaluate the effects of isolation and handling of the cells. In the second experiment, CML was recorded after stimulation of 1.25×10^6 cells of the same batch with 8×10^{-7} M PMA. The CML response of neutrophils (expressed in mV) was measured using a luminometer Bio-Orbit 1251 at 37.8 °C and the maximum CML value (CML peak value) recorded during 10 min of CML monitoring was considered as the final result of the measurement.

Statistical analysis

The peak CML value per measurement was used for the calculation of mean values (±SD). ANOVA with Tukey's *post hoc* test and a *t*-test for paired data were performed with the

GRAPHPAD INSTAT 3.05 (GraphPad™ Software, San Diego, CA, USA). The level of significance was set at 0.05.

RESULTS

Figure 1 shows the individual peak CML values for all horses at T0 before treatment. The CML response of PMA stimulated neutrophils is compared with that of nonstimulated neutrophils (basal value). The results show that there is a significant increase in the CML response after stimulation with PMA (to 12 times the basal value). The mean CML value for unstimulated cells was 125 ± 87 mV, significantly different from the mean CML value (1473 ± 479 mV) measured for stimulated neutrophils. For the following results focused in this study, the basal CML value measured for the nonstimulated neutrophils was subtracted from the CML value measured with PMA activation.

Time evolution of the effects of the in vivo *administration of ACP or PTZ on the CML response of isolated neutrophils*

Figure 2 shows the time-dependent CML response of neutrophils for each horse after ACP (Fig. 2a) or PTZ (Fig. 2b) administration. For all horses, a decrease of the CML response and thus of the ROS production after PHE administration was observed. However, the time to reach the maximal inhibition differed. In some cases, there was a very rapid and important decrease of CML, persisting until 5 h especially in the ACP group (horses 1, 2 and 3), in other cases for both groups, the inhibitory effect appeared later, often after a slight increase at 0.5 and 1 h. Generally, it appeared that ACP exerted a more pronounced inhibitory effect as compared with PTZ.

Time-dependent inhibition of CML by ACP or PTZ

Table 1 shows the mean (±SD) relative values of CML response at each sampling time for the two groups of horses. There was a

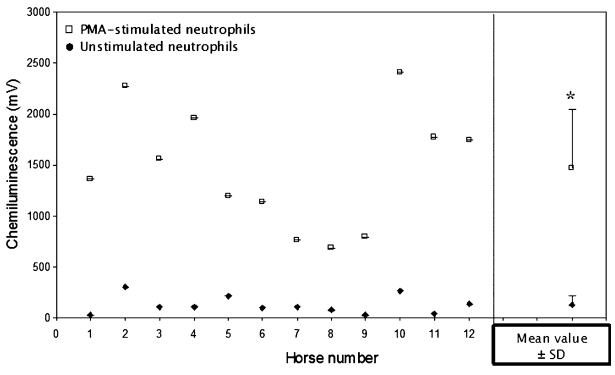

Fig. 1. Individual luminol-enhanced chemiluminescence values (in mV) produced by neutrophils isolated from 12 horses: response of the cells to an *in vitro* stimulation by 8.10^{-7} M phorbol myristate acetate (□) compared with the cell response without stimulation (●). The neutrophils were isolated from blood drawn before the phenothiazine (PHE) injection (T0). For each condition, the calculated mean values (±SD) for the 12 horses are presented on the right side of the figure. *$P < 0.05$.

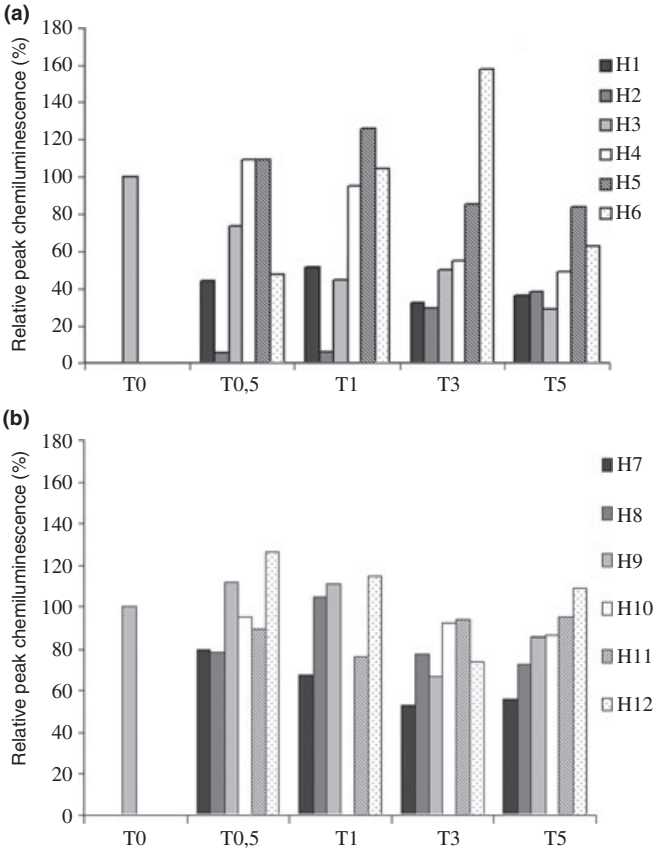

Fig. 2. Time evolution of individual relative peak chemiluminescence produced by 1.25×10^6 equine neutrophils stimulated with 8.10^{-7} M phorbol myristate acetate in 12 horses. (a) Six horses (horses 1–6) received 0.1 mg/kg acepromazine i.v. (b) Six horses (horses 7–12) received 0.1 mg/kg promethazine. The peak CL response of neutrophils measured at T0 [before acepromazine (ACP) or promethazine (PTZ) administration] was taken as 100% for each horse. Blood sampling times: T0: before ACP or PTZ administration, T0.5, T1, T3, T5: respectively 0.5, 1, 3 and 5 h after drug administration.

Table 1. Time evolution of mean peak value of chemiluminescence produced by 1.25×10^6 equine neutrophils stimulated with 8.10^{-7} M phorbol myristate acetate in 12 horses

Table 1	T0.5	T1	T3	T5
Group A (ACP) $n = 6$	65 ± 41%	72 ± 45%	68 ± 48%	51 ± 20%*
Group B (PTZ) $n = 6$	97 ± 20%	90 ± 22%	76 ± 15%*	84 ± 18%

Mean peak chemiluminescence values (±SD) are expressed in % of T0 value.
ACP, acepromazine; PTZ, promethazine.
*$P < 0.05$ vs. T0.

significant inhibition of ROS production in the ACP group (49% inhibition) at 5 h and in the PTZ group (24% inhibition) at 3 h. In the ACP group, the mean neutrophil CML response was

829 ± 467 mV at 5 h compared with 1582 ± 449 mV at T0 ($P < 0.05$). For PTZ at 3 h the CML value was 1110 ± 741 mV compared with 1364 ± 713 mV at T0 ($P < 0.05$).

DISCUSSION

The decrease of CML produced by equine neutrophils stimulated by PMA was already demonstrated by our team *in vitro* for ACP. Other PHE or thioxanthenes also possess similar antioxidant properties as demonstrated with polymorphonuclear leucocytes (Hadjimitova *et al.*, 1999, 2003).

In *in vitro* models, ACP at 10^{-5} M showed an inhibitory capacity of the ROS production by neutrophils of about 50% whereas at 10^{-4} M, the inhibition was complete (Serteyn *et al.*, 1999). In our study, we also observed a decrease of ROS production by equine neutrophils after a single i.v. administration of 0.1 mg/kg PHE, a dose which is estimated to result in a plasma concentration of 4.6×10^{-6} M and 5.3×10^{-6} M for ACP and PTZ respectively. This comparison indicates that the *in vitro* and *in vivo* effects are comparable.

To our knowledge, it is the first time that the antioxidant properties of an *in vivo* administration of PHE were demonstrated *ex vivo* on isolated neutrophils. This approach showed also the significant individual variability of the neutrophil response to stimulation.

Luminol enhanced CML measures the global production of ROS, including highly pro-oxidant species such as hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$), which are more reactive with luminol than other ROS species such as superoxide anion radicals ($O_2{\cdot}^-$) or hydrogen peroxide (H_2O_2). The variable inhibition of the neutrophil response to stimulation with PMA was therefore not unexpected. However, it implies that the CML response at the different sampling times after the PHE administration in a horse would need to be compared with a control sample from the same horse before administration of the drug. The important individual variability of the CML response also explains the rather high standard deviation of the mean CML values and the limited or lacking statistical significance.

The pharmacokinetic parameters of ACP in horse were reported to be variable within the same experimental protocol (Ballard *et al.*, 1982; Hashem & Keller, 1993; Marroum *et al.*, 1994), which could also explain the variability of the CML response. The diet, training status and level of anxiety of an individual animal during the month preceding the study could also have influenced the results. It has been shown that stress and intensity of exercise influenced the oxidative metabolism of human and equine neutrophils (Pyne *et al.*, 2000; Art *et al.*, 2006). This implies that in *in vivo* studies the influence of catecholamines on neutrophil functions such as trafficking and activation needs to be taken into account (Abraham *et al.*, 1999; Altenburg *et al.*, 2000).

Antioxidant effects of PHE were always found more important in a cellular model than in a cell-free system (Serteyn *et al.*, 1999; Hadjimitova *et al.*, 2004). This observation could show that PHE have an intracellular target, which enhances their fundamental scavenging effect on ROS. MPO, a specific haeme enzyme of the azurophilic granules of the neutrophils which participates in pathogen destruction within the phagocytic vacuole, is specifically responsible for the production of a potent oxidant agent, HOCl synthetized from chloride anion (Cl^-) and H_2O_2 and could be this specific intracellular target for PHE. The reaction of PHE with MPO in a cellular model can result in the establishment of a PHE-derived cationic radical, biologically more active (nucleic acids and protein covalent binding) and with more pronounced scavenging capacities than the mother compounds (van Zyl *et al.*, 1990; Kelder *et al.*, 1991). Finally, according to some authors (Ordway *et al.*, 2002; Kristiansen *et al.*, 2007), PHE are concentrated in some tissues like the lungs and in leucocytes. In our model, the PHE with an initial lower plasma concentration had antioxidant effects comparable to those observed in *in vitro* cellular models. The neutrophil isolation steps included elimination of plasma and red blood cells which implicate the absence of any free drug in the buffered solution, but the anti-oxidant effects persisted. Thus, we hypothesized that the PHE molecules were linked to the neutrophil membrane, but kept their ROS scavenging functions, or were inside the cell where they could inhibit the Ca^{2+}/calmodulin pathway of leucocyte activation or reacted with MPO to produce the more active cationic radicals. These hypotheses need to be confirmed *in vitro* for ACP and PTZ before a final conclusion can be drawn on the mechanism of antioxidant action observed in this study.

The vasodilatory effect of the drugs is considered as interesting for the treatment of equine laminitis and to increase muscular blood flow during anaesthesia. The antioxidant effect demonstrated in this study could be relevant in the prevention of these pathologies where ROS are implicated. Recently, some authors have shown that ROS were implicated in the endotoxin-induced impairment of β-adrenoceptor-mediated vasodilation in equine digital veins (Mallem *et al.*, 2003, 2006). In agreement with these authors, our results suggest a synergistic effect of vasodilator and antioxidant properties of ACP that could be beneficial for the treatment of laminitis in horses. Compared with ACP, PTZ seems to be less efficient on the activity of stimulated neutrophils. But PTZ shows less cardiovascular depression in horses as it does in other species; this molecule could therefore be interesting for horses with severe cardiovascular failure, when an increase of ROS production occurs and the use of ACP would not be optimal. PTZ has also a more pronounced anti-histaminic effect than ACP, but possesses anti-inflammatory properties (Gusdon *et al.*, 1972; Rychlik *et al.*, 1988; Molnar *et al.*, 1992) and could be interesting particularly for horses with severe endotoxic shock, because of its limited vasodilating effect (Covert *et al.*, 1988; Brown & Eckberg, 1997; Péters *et al.*, 2002).

In conclusion, the inhibition by ACP and PTZ of the CML produced by PMA-stimulated equine neutrophils is the first confirmation of the antioxidant properties following an *in vivo* administration of PHE. Therefore, PHE could have interesting therapeutic perspectives in inflammatory diseases of horses implicating neutrophil activation and ROS production.

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REFERENCES

Abraham, E., Kaneko, D.J. & Shenkar, R. (1999) Effects of endogenous and exogenous catecholamines on LPS-induced neutrophil trafficking and activation. *American Journal of Physiology*, **276,** L1–L8.

Aftab, D.T., Ballas, L.M., Loomis, C.R. & Hait, W.N. (1991) Structure-activity relationships of phenothiazines and related drugs for inhibition of protein kinase C. *Molecular Pharmacology*, **40,** 798–805.

Altenburg, S.P., Paixao e Silva, S., Ventura, D.G., Gomes, R.N., Bozza, P.T. & Castro-Faria-Neto, H.C. (2000) A role for adrenoceptors in the regulation of pleural neutrophilia induced by LPS. *Journal of Neuroimmunology*, **111,** 15–22.

Art, T., Franck, T., Gangl, M., Votion, D., Kohnen, S., Deby-Dupont, G. & Serteyn, D. (2006) Plasma concentrations of myeloperoxidase in endurance and 3-day event horses after a competition. *Equine Veterinary Journal. Supplement*, **36,** 298–302.

Babu, A.S. & Gowda, T.V. (1995) Effects of chlorpromazine on the enzymatic and toxic properties of the most basic phospholipase A2 from *Vipera russelli* snake venom. *Inflammation Research*, **44,** 340–344.

Ballard, S., Shults, T., Kownacki, A.A., Blake, J.W. & Tobin, T. (1982) The pharmacokinetics, pharmacological responses and behavioral effects of acepromazine in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **5,** 21–31.

Benbarek, H., Deby-Dupont, G., Deby, C., Caudron, I., Mathy-Hartert, M., Lamy, M. & Serteyn, D. (1996) Experimental model for the study by chemiluminescence of the activation of isolated equine leucocytes. *Research in Veterinary Science*, **61,** 59–64.

Blackwood, R.A. & Hessler, R.J. (1995) Effect of calcium on phenothiazine inhibition of neutrophil degranulation. *Journal of Leukocyte Biology*, **58,** 114–118.

Bleeker, M.W., Netea, M.G., Kullberg, B.J., Van der Ven-Jongekrijg, J. & Van der Meer, J.W. (1997) The effects of dexamethasone and chlorpromazine on tumour necrosis factor-alpha, interleukin-1 beta, interleukin-1 receptor antagonist and interleukin-10 in human volunteers. *Immunology*, **91,** 548–552.

Boraschi, D., Villa, L., Ghiara, P., Tagliabue, A., Mengozzi, M., Solito, E., Parente, L., Silvestri, S., Van Damme, J. & Ghezzi, P. (1991) Mechanism of acute toxicity of IL-1 beta in mice. *European Cytokine Network*, **2,** 61–67.

Boyum, A. (1976) Isolation of lymphocytes, granulocytes and macrophages. *Scandinavian Journal of Immunology*, Suppl **5,** 9–15.

Brown, T.E. & Eckberg, D.L. (1997) Promethazine affects autonomic cardiovascular mechanisms minimally. *Journal of Pharmacology and Experimental Therapeutics*, **282,** 839–844.

Brufani, M., Cesta, M.C., Filocamo, L., Lappa, S., Marta, M., Pomponi, M., Meroni, C. & Pagella, P.G. (1992) Synthesis of phenothiazine derivatives as potential inhibitors of phospholipase C. *Il Farmaco*, **47,** 585–597.

Chen, B.C., Hsieh, S.L. & Lin, W.W. (2001) Involvement of protein kinases in the potentiation of lipopolysaccharide-induced inflammatory mediator formation by thapsigargin in peritoneal macrophages. *Journal of Leukocyte Biology*, **69,** 280–288.

Clancy, K.D., Lorenz, K., Dries, D., Gamelli, R.L. & Hahn, E.L. (2000) Chlorpromazine modulates cytokine expression in the liver and lung after burn injury and endotoxemia. *Journal of Trauma: Injury, Infection, and Critical Care*, **48,** 215–222 discussion 222–213.

Covert, R.F., Drummond, W.H., Webb, I.B. & Gimotty, P.A. (1988) Pulmonary and systemic vascular response to promethazine in conscious lambs. *Pediatric Cardiology*, **9,** 137–141.

Dalla Libera, A., Scutari, G., Boscolo, R., Rigobello, M.P. & Bindoli, A. (1998) Antioxidant properties of clozapine and related neuroleptics. *Free Radical Research*, **29,** 151–157.

Delieu, J.M., Badawoud, M., Williams, M.A., Horobin, R.W. & Duguid, J.K. (2001) Antipsychotic drugs result in the formation of immature neutrophil leucocytes in schizophrenic patients. *Journal of Psychopharmacology*, **15,** 191–194.

Easmon, C.S., Cole, P.J., Williams, A.J. & Hastings, M. (1980) The measurement of opsonic and phagocytic function by luminol-dependent chemiluminescence. *Immunology*, **41,** 67–74.

Elferink, J.G. (1979) Chlorpromazine inhibits phagocytosis and exocytosis in rabbit polymorphonuclear leukocytes. *Biochemical Pharmacology*, **28,** 965–968.

Gadina, M., Bertini, R., Mengozzi, M., Zandalasini, M., Mantovani, A. & Ghezzi, P. (1991) Protective effect of chlorpromazine on endotoxin toxicity and TNF production in glucocorticoid-sensitive and glucocorticoid-resistant models of endotoxic shock. *Journal of Experimental Medicine*, **173,** 1305–1310.

Ghezzi, P., Garattini, S., Mennini, T., Bertini, R., Delgado Hernandez, R., Benigni, F., Sacco, S., Skorupska, M., Mengozzi, M., Latini, R., Kurosaki, M., Lombet, A., Fradin, A., Bonnet, J., Rolland, Y. & Brion, J.D. (1996) Mechanism of inhibition of tumor necrosis factor production by chlorpromazine and its derivatives in mice. *European Journal of Pharmacology*, **317,** 369–376.

Grabski, R., Dewit, J., De Braekeleer, J., Malicka-Blaskiewicz, M., De Baetselier, P. & Verschueren, H. (2001) Inhibition of T-cell invasion across cultured fibroblast monolayers by phenothiazine-related calmodulin inhibitors: impairment of lymphocyte motility by trifluoperazine and chlorpromazine, and alteration of the monolayer by pimozide. *Biochemical Pharmacology*, **61,** 1313–1317.

Gusdon, J.P. Jr, Moore, V.L., Myrvik, Q.N. & Holyfield, P.A. (1972) Promethazine-HCl as an immunosuppressant. *Journal of Immunology*, **108,** 1340–1344.

Hadjimitova, V., Traykov, T., Goliysky, P. & Ribarov, S. (1999) Effect of some psychotropic drugs on the activated macrophage-induced luminol-dependent chemiluminescence. *Pharmacology and Toxicology*, **84,** 170–173.

Hadjimitova, V., Traykov, T., Mileva, M. & Ribarov, S. (2002) Effect of some psychotropic drugs on luminol-dependent chemiluminescence induced by $O2^{-}$, *OH, HOCl. *Zeitschrift für Naturforschung. Section C. Biosciences*, **57,** 1066–1071.

Hadjimitova, V., Bakalova, R., Traykov, T., Ohba, H. & Ribarov, S. (2003) Effect of phenothiazines on protein kinase C- and calcium-dependent activation of peritoneal macrophages. *Cell Biology and Toxicology*, **19,** 3–12.

Hadjimitova, V., Traykov, T., Bakalova, R., Petrova, V., Lambev, I., Ohba, H., Ishikawa, M. & Baba, Y. (2004) Chemiluminescent analysis of the antioxidant and immunomodulation effects of several psychotropic drugs on peritoneal macrophages. *Luminescence*, **19,** 319–321.

Hashem, A. & Keller, H. (1993) Disposition, bioavailability and clinical efficacy of orally administered acepromazine in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **16,** 359–368.

Hurley, D.J., Parks, R.J., Reber, A.J., Donovan, D.C., Okinaga, T., Vandenplas, M.L., Peroni, J.F. & Moore, J.N. (2006) Dynamic changes in circulating leukocytes during the induction of equine laminitis with black walnut extract. *Veterinary Immunology and Immunopathology*, **110,** 195–206.

Ingle-Fehr, J.E. & Baxter, G.M. (1999) The effect of oral isoxsuprine and pentoxifylline on digital and laminar blood flow in healthy horses. *Veterinary Surgery*, **28,** 154–160.

Jain, M.K., Yu, B.Z., Rogers, J., Ranadive, G.N. & Berg, O.G. (1991) Interfacial catalysis by phospholipase A2: dissociation constants for calcium, substrate, products, and competitive inhibitors. *Biochemistry*, **30,** 7306–7317.

Johnston, G.M., Eastment, J.K., Wood, J.L.N. & Taylor, P.M. (2002) The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of phases 1 and 2. *Veterinary Anaesthesia and Analgesia*, **29,** 159–170.

Jones, H.P., Ghai, G., Petrone, W.F. & McCord, J.M. (1982) Calmodulin-dependent stimulation of the NADPH oxidase of human neutrophils. *Biochimica et Biophysica Acta*, **714,** 152–156.

Kelder, P.P., de Mol, N.J., BA, T.H. & Janssen, L.H. (1991) Metabolic activation of chlorpromazine by stimulated human polymorphonuclear leukocytes. Induction of covalent binding of chlorpromazine to nucleic acids and proteins. *Chemico-Biological Interactions*, **79,** 15–30.

Kiku, Y., Matsuzawa, H., Ohtsuka, H., Terasaki, N., Fukuda, S., Kon-Nai, S., Koiwa, M., Yokomizo, Y., Sato, H., Rosol, T.J., Okada, H. & Yoshino, T.O. (2002) Effects of chlorpromazine, pentoxifylline and dexamethasone on mRNA expression of lipopolysaccharide-induced inflammatory cytokines in bovine peripheral blood mononuclear cells. *Journal of Veterinary Medical Science*, **64,** 723–726.

Kowalski, J., Blada, P., Kucia, K., Madej, A. & Herman, Z.S. (2001) Neuroleptics normalize increased release of interleukin-1 beta and tumor necrosis factor-alpha from monocytes in schizophrenia. *Schizophrenia Research*, **50,** 169–175.

Kristiansen, J.E., Hendricks, O., Delvin, T., Butterworth, T.S., Aagaard, L., Christensen, J.B., Flores, V.C. & Keyzer, H. (2007) Reversal of resistance in microorganisms by help of non-antibiotics. *Journal of Antimicrobial Chemotherapy*, **59,** 1271–1279.

Loftus, J.P., Belknap, J.K., Stankiewicz, K.M. & Black, S.J. (2007) Laminar xanthine oxidase, superoxide dismutase and catalase activities in the prodromal stage of black-walnut induced equine laminitis. *Equine Veterinary Journal*, **39,** 48–53.

Mallem, M.Y., Gogny, M., Gautier, F., Bucas, V. & Desfontis, J.C. (2003) Evaluation of beta3-adrenoceptor-mediated relaxation in intact and endotoxin-treated equine digital veins. *American Journal of Veterinary Research*, **64,** 708–714.

Mallem, M.Y., Le Coz, O., Thuleau, A., Desfontis, J.C. & Gogny, M. (2006) Reactive oxygen species mediate endotoxin-induced impairement of beta-adrenoceptors-mediated vasodilatation in equine digital veins. *Journal of Veterinary Pharmacology and Therapeutics*, **29,** 234–235.

Marntell, S., Nyman, G., Funkquist, P. & Hedenstierna, G. (2005) Effects of acepromazine on pulmonary gas exchange and circulation during sedation and dissociative anaesthesia in horses. *Veterinary Anaesthesia and Analgesia*, **32,** 83–93.

Marroum, P.J., Webb, A.I., Aeschbacher, G. & Curry, S.H. (1994) Pharmacokinetics and pharmacodynamics of acepromazine in horses. *American Journal of Veterinary Research*, **55,** 1428–1433.

Matthews, N., Franklin, R.J. & Kendrick, D.A. (1995) Structure-activity relationships of phenothiazines in inhibiting lymphocyte motility as determined by a novel flow cytometric assay. *Biochemical Pharmacology*, **50,** 1053–1061.

Mengozzi, M., Fantuzzi, G., Faggioni, R., Marchant, A., Goldman, M., Orencole, S., Clark, B.D., Sironi, M., Benigni, F. & Ghezzi, P. (1994) Chlorpromazine specifically inhibits peripheral and brain TNF production, and up-regulates IL-10 production, in mice. *Immunology*, **82,** 207–210.

Molnar, J., Mandi, Y., Regely, K., Tarnoky, K. & Nakamura, M.J. (1992) Inhibition of biological effects of endotoxins by phenothiazines. *In Vivo*, **6,** 205–209.

Ohtsuka, H., Higuchi, T., Matsuzawa, H., Sato, H., Takahashi, K., Takahashi, J. & Yoshino, T.O. (1997) Inhibitory effect on LPS-induced tumor necrosis factor in calves treated with chlorpromazine or pentoxifylline. *Journal of Veterinary Medical Science*, **59,** 1075–1077.

Ordway, D., Viveiros, M., Leandro, C., Arroz, M.J. & Amaral, L. (2002) Intracellular activity of clinical concentrations of phenothiazines including thioridiazine against phagocytosed *Staphylococcus aureus*. *International Journal of Antimicrobial Agents*, **20,** 34–43.

Péters, F., Pequito, M., Deby-Dupont, G. & Serteyn, D. (2002) Comparison of sedation and hypotension produced by acepromazine and by rising doses of promethazine in the horse. *Proceedings Association of Veterinary Anaesthetists Autumn Meeting*, Vol. 30, p. 41, 26–28 September, Heraklion, Greece.

Pollmacher, T., Haack, M., Schuld, A., Kraus, T. & Hinze-Selch, D. (2000) Effects of antipsychotic drugs on cytokine networks. *Journal of Psychiatric Research*, **34,** 369–382.

Pycock, J.F., Allen, W.E. & Morris, T.H. (1987) Rapid, single-step isolation of equine neutrophils on a discontinuous Percoll density gradient. *Research in Veterinary Science*, **42,** 411–412.

Pyne, D.B., Smith, J.A., Baker, M.S., Telford, R.D. & Weidemann, M.J. (2000) Neutrophil oxidative activity is differentially affected by exercise intensity and type. *Journal of Science and Medicine in Sport*, **3,** 44–54.

Radenovic, L. & Kartelija, G. (2004) Effect of chlorpromazine on human and murine intracellular carboxylesterases. *Biochemistry*, **69,** 381–386.

Richter, J. (1990) Lymphotoxin induces secretion of granule proteins from adherent neutrophils: possible role of intracellular free calcium. *Journal of Leukocyte Biology*, **47,** 506–513.

Riggs, L.M., Franck, T., Moore, J.N., Krunkosky, T.M., Hurley, D.J., Peroni, J.F., de la Rebiere, G. & Serteyn, D.A. (2007) Neutrophil myeloperoxidase measurements in plasma, laminar tissue, and skin of horses given black walnut extract. *American Journal of Veterinary Research*, **68,** 81–86.

Rychlik, G., Rychlik, E. & Wasik, M. (1988) Influence of promethazine on immune reactions. I. Characterization of promethazine effects on natural killer cell-mediated cytotoxicity. *Immunopharmacology*, **15,** 117–122.

Serteyn, D., Benbarek, H., Deby-Dupont, G., Grulke, S., Caudron, I., Deby, C. & Lamy, M. (1999) Effects of acepromazine on equine polymorphonuclear neutrophil activation: a chemiluminescence study. *Veterinary Journal*, **157,** 332–335.

Serteyn, D., Gangl, M., Grulke, S., Péters, F., Deby-Dupont, G. & Lamy, M. (2000) Antilipoperoxidant properties of acepromazine. *Proceedings 7th World Congress of Veterinary Anaesthesia*, p. 50, 20–23 September, Berne, Switzerland.

Serteyn, D., Deby-Dupont, G., Péters, F. & Deby, C. (2001) Acepromazine modulates the cell differentiation: in vitro experiments on monocytes stimulated by *Chlamydia pneumoniae* toxins. *Proceedings Association of Veterinary Anaesthetists Spring Meeting*, p. 46, 3–4 March, Uppsala, Sweden.

Serteyn, D., Deby-Dupont, G., Péters, F. & Deby, C.. (2002) Comparative study of the effects of acepromazine and structuraly parent compounds on the TNF-a release by monocytes stimulated by Chlamydia Pneumoniae. *Proceedings Association of Veterinary Anaesthetists Autumn Meeting*, p. 35, 26–28 September, Heraklion, Greece.

Slater, M.R., Hood, D.M. & Carter, G.K. (1995) Descriptive epidemiological study of equine laminitis. *Equine Veterinary Journal*, **27,** 364–367.

Steele, T.A. & Brahmi, Z. (1988) Chlorpromazine inhibits human natural killer cell activity and antibody-dependent cell-mediated cytotoxicity. *Biochemical and Biophysical Research Communications*, **155,** 597–602.

Szuster-Ciesielska, A., Slotwinska, M., Stachura, A., Marmurowska-Michalowska, H. & Kandefer-Szerszen, M. (2004) Neuroleptics

modulate cytokine and reactive oxygen species production in blood leukocytes of healthy volunteers. *Archivum Immunologiaeet Therapiae Experimentalis*, **52,** 59–67.

Traykov, T., Hadjimitova, V., Goliysky, P. & Ribarov, S. (1997) Effect of phenothiazines on activated macrophage-induced luminol-dependent chemiluminescence. *General Physiology and Biophysics*, **16,** 3–14.

Watanabe, T., Hashimoto, Y., Teramoto, T., Kume, S., Naito, C. & Oka, H. (1986) Calmodulin-independent inhibition of platelet phospholipase A2 by calmodulin antagonists. *Archives of Biochemistry and Biophysics*, **246,** 699–709.

Wightman, P.D., Dahlgren, M.E., Hall, J.C., Davies, P. & Bonney, R.J. (1981) Identification and characterization of a phospholipase C activity in resident mouse peritoneal macrophages. Inhibition of the enzyme by phenothiazines. *Biochemical Journal*, **197,** 523–526.

van Zyl, J.M., Basson, K., Kriegler, A. & van der Walt, B.J. (1990) Activation of chlorpromazine by the myeloperoxidase system of the human neutrophil. *Biochemical Pharmacology*, **40,** 947–954.