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The Effect of Prednisolone on Canine Neutrophil Function: In Vivo and in Vitro Studies

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Trowald-Wigh G, Håkansson L, Johannisson A, Edqvist LE: The effect of prednisolone on canine neutrophil function: In vivo and in vitro studies. Acta vet. scand. 1998, 39, 201-213. – The in vivo effect of a therapeutic dose of prednisolone on canine neutrophil adherence, random migration, chemotaxis, phagocytosis of IgG and C3b opsonized yeast cells, chemiluminescence, Fc- and CR3-receptor expression was investigated. Prednisolone was also added in vitro to neutrophils as isolated cells and in whole blood. In the in vivo study, prednisolone increased the IgG mediated ingestion of yeast cells and the number of activated neutrophils in the phagocytosis assay, while flow cytometric investigation of the IgG-receptor FcγRIII with a monoclonal antibody showed similar expression before, during and after treatment. Prednisolone also increased the ingestion of C3b-opsonized yeast cells, while the expression of CR3-receptors (CD11b CD18) measured by flow cytometry was unchanged. Chemiluminescence and the chemotactic response towards zymosan activated serum were increased, while adherence to nylon wool was decreased. The in vitro studies revealed that prednisolone had no or a dampening effect on neutrophils in cell suspensions. Adherence as well as IgG mediated ingestion was decreased at the highest prednisolone concentration (800 ng/ml) in whole blood. The present study suggests that the part of the antiinflammatory effect of corticosteroids mediated through their influence on neutrophils, besides reduced adherence, may be exerted by increased clearance of microorganisms and IgG-complexes through an elevated functional capacity.

adhesion; migration; chemotaxis; phagocytosis; FcγIII-receptor-expression; CR3-receptor expression; corticosteroids.

Introduction

Corticosteroids influence a multitude of physiological and pathological processes and are therefore widely used in the therapy of a wide spectrum of inflammatory and autoimmune diseases. Many in vivo and in vitro studies have been performed to investigate the effect of corticosteroids on granulopoiesis and neutrophil function. Hydrocortisone stimulates granulopoiesis in bone marrow cultures (*Barr et al.* 1983, *Inada et al.* 1988, *Motomura et al.* 1983, *Suda et al.* 1983), while experiments on the influence of corticosteroids on the function of neutrophils give conflicting results. *Hirsch & Church* (1961) found that high doses of cortisone in vivo did not affect phagocytic and bactericidal capacity of rabbit neutrophils. *Roth & Kaeberle* (1981) investigated the effects of one single dose of dexamethasone on bovine neutrophils and found enhanced random migration under agarose, impaired ingestion of Staphylococcus aureus, suppressed NBT-reduction, and chemiluminescence. *Levine et al.* (1981) found

that methylprednisolone at high concentrations decreased chemiluminescence in vitro, while other investigators found increased neutrophil function after corticosteroid treatment in vivo (*Guelfi et al.* 1985, *Phillips et al.* 1987, *Morris et al.* 1988, *Kuzniar et al.* 1991). The contradictory results of the studies on corticosteroid influence on neutrophils both in vivo and in vitro are probably due to the use of different drugs, different ways of administration of the drug, different doses, and varying assays for studying neutrophil functions.

The aim of this study was to gain knowledge of how a therapeutic dose of prednisolone, given in a therapeutic way, influences neutrophil function both in vivo and in vitro.

Therefore, prednisolone was administered to dogs and the influence on neutrophil adherence, chemotaxis, phagocytosis, chemiluminescence, and expression of CD16, CD11b and CD18 was investigated during the treatment period and immediately thereafter. In order to evaluate whether the obtained results of prednisolone administration on neutrophil function were due to a direct effect of prednisolone on neutrophils, in vitro studies were performed where neutrophils were incubated with different concentrations of prednisolone in whole blood and as isolated cells.

Materials and methods

Blood sampling

Blood was collected from the cephalic vein into heparinized tubes (Vacutainer system, Becton Dickinson, Meyland, France) for the neutrophil function tests and in EDTA tubes (Becton Dickinson) for determination of hemoglobin (Hb), white blood cell count (WBC), and differential leucocyte count.

In vivo study

Six healthy beagle dogs, 3 females and 3 males, 10-29 months of age, were included. The dogs had lived in the same environment for at least 1 month prior to the experiment. The dogs were their own controls, and blood samples were analyzed before (C1: mean of 2 samples) and after (C2: one drugfree week, C3: 2 drugfree weeks) the period of drug administration. Prednisolone tablets (5 mg) (Nordvacc, Skärholmen, Stockholm, Sweden) were administered in a dose of 1 mg/kg, for 3 weeks (T 1-3) and then the dose was decreased during 2 weeks to 0.7 mg/kg (T4) and 0.3 mg/kg (T5), respectively. Blood sampling was performed once a week. The daily dose was divided equally between morning and afternoon administrations, and the morning dose was administered one hour before blood sampling. Besides the neutrophil function tests, analysis of Hb, WBC, differential leucocyte count and serum alkaline phosphatase were performed. At each experiment, blood was drawn from one healthy dog, and neutrophil function was analyzed together with the experiment group as a control of the methods.

In order to study the expression of Fc-receptors during treatment, prednisolone (1mg/kg) was administered to 2 healthy beagles (2 and 3 years old) according to the same routine as previously described, and one dog was used as control (blood was analyzed together with the experimental dogs). Blood was drawn on 8 occasions; before the experiment (C1: mean of 2 samples), in each of the 3 weeks of 1mg/kg treatment (T1-T3), while tapering the dose (T4-5) and 2 weeks after terminated treatment (C2). Blood was drawn one hour after the morning dose and WBC, differential count and adhesion assay were performed. Phagocytosis was performed at time points C1, T1, T2, T3 and T4.

In vitro studies

Prednisolone in whole blood: Blood was drawn from 6 healthy beagle dogs (mean age 3

years). Prednisolone 21-acetate (Sigma Chemical Company, St. Louis, USA), was added to whole blood immediately after blood sampling. Before cell-separation, the blood was incubated for 30 min at 37 °C in 5 different tubes, one control sample without prednisolone and 4 tubes with prednisolone at concentrations of 100, 200, 400 and 800 ng/ml blood (277, 554, 1104 and 2219 nmol/l), respectively. Neutrophils were isolated and their adherence, chemotaxis, phagocytosis, and chemiluminescence were assayed.

Prednisolone with isolated neutrophils: Blood was drawn from 10 healthy beagle dogs (mean age 2 years). Neutrophils were isolated and diluted to the final cell concentration (5×10^6 and 6×10^5). Prednisolone 21-acetate was added to the cell suspension samples to final concentrations of 50, 100 and 200 ng/ml (138, 277 and 544 nmol/l). Samples without prednisolone served as controls. Cell samples were incubated at 37 °C for 30 min, whereafter neutrophil chemotaxis, phagocytosis, and chemiluminescence were assayed.

Isolation of neutrophils

Neutrophils were isolated from heparinized blood by a dextran sedimentation technique according to a method previously described (*Trowald-Wigh & Thoren-Tolling* 1990 a and b).

Measurement of neutrophil function

Adhesion. Adhesion of neutrophils was assayed using a slight modification (*Trowald-Wigh et al.* 1991) of the method described by *Mac Gregor et al.* (1974). The blood was allowed to pass through columns of scrubbed nylon fibers. Adherence was expressed as the concentration of neutrophils after passage in relation to the original concentration of neutrophils (%).

Random migration and chemotaxis. Random migration and chemotaxis of neutrophils were assayed according to a modified Boyden chamber technique (*Wilkinsson* 1974). Random migration was defined as migration of neutrophils through a filter against buffer and chemotaxis as the migration of neutrophils versus casein (1g/l) and zymosan activated canine serum (10%) in buffer (Trowald-Wigh and Thoren-Tolling 1990b). Migration was assayed according to the leading front method (*Zigmond & Hirsch* 1973).

Phagocytosis

The phagocytosis method is described in detail in earlier works (*Trowald-Wigh & Thoren-Tolling* 1990a). Neutrophils were incubated at 37 °C with serum(C3b)-opsonized and anti yeast IgG-opsonized yeast-cells, respectively, on 3 spot glass slides. Phagocytosis was interrupted after 35 min and trypan blue was added. Trypan blue extinguishes the fluorescence outside the neutrophil while the ingested yeast cells retain their fluorescence (*Hed* 1977). In this assay it is possible to distinguish between adherent yeast cells and ingested and count the number of ingested yeast cells/100 neutrophils. The percentage of activated neutrophils, i.e. neutrophils that have either adhered or ingested yeast cells, was also assessed.

Chemiluminescence

Neutrophils were mixed with serum opsonized zymosan as described in an earlier work (*Trowald-Wigh & Thoren-Tolling* 1990b). In this assay, luminol (Sigma St. Louis, USA) (0.025 mg/ml) was used as amplifier. Chemiluminescence was registered as RLU (relative light units) until the peak value was obtained and the curve declined.

Expression of Fc γreceptor III and complement receptor 3 of neutrophils in the presence and absence of prednisolone

Blood was drawn from the 2 dogs receiving prednisolone and the control dog at C1, C2 and T1-5. Neutrophils were frozen in RPMI 1640 medium with 10% foetal calf serum and 10% DMSO immediately after separation and stored at –70 °C until analysis. Later, FcγRIII (CD16), CD11b and CD18-receptor expression on neutrophils in the frozen samples from all experiments and controls were analyzed at the same time to avoid differences in staining between sampling occasions.

Monoclonal antibodies

The following murine anti-human monoclonal antibodies were used in this study: FITC-conjugated anti-CD16 (Immunotech S.A. Marseille, France), FITC-conjugated anti-CD18 (Dakopatts AB, Glostrup, Denmark) and FITC-conjugated anti-CD11b (Immunotech S.A. Marseille, France). An irrelevant mouse monoclonal (X927, Dakopatts AB) served as negative control.

Staining with FITC conjugated antibodies

Cells were thawed in a 20 °C waterbath and spun down at 1000 rpm. The freezing medium was removed and cells were washed once with RPMI containing 10% FCS and 5×10^5 cells were stained for 45 min on ice with 10 μl antibody, diluted 1:10 in RPMI with 10% FCS. Cells were washed once and resuspended in 500 μl PBS.

Flow cytometry

Flow-cytometric analyses were conducted with a FACStarPLUS flow cytometer (Becton Dickinson Immunocytometry Systems) with standard optical equipment. As light source, a 488 nm Argon ion-laser was used, running at 200 mW. Forward light scatter, orthogonal light scatter and FITC fluorescence of 10000 events from each sample were collected in list-mode using the FACStarPLUS Research software version 2.01 (BD). Forward light scatter and orthogonal light scatter were collected using linear amplification, FITC fluoroscence was collected using logarithmic amplification. Offline analysis was conducted with LYSYS version 1.62 (BD). A granulocyte gate was set on the FSC-SSC dot-plot, and the distributions of FITC-fluorescence were investigated for gated cells. Peak values of FITC-distributions were calculated for each sample.

Prednisolone in serum

The serum concentration of prednisolone/cortisol was measured by the use of Amerlite Cortisol assay (Amersham Sweden AB Solna) which is a competitive immunoassay technique.

The use of Amerlite Cortisol assay to estimate the concentration of prednisolone in serum is based on the cross-reaction between prednisolone and cortisol. According to the manufacturer, the cross-reaction of prednisolone in the cortisol assay is 25%. We determined the cross-reaction to be higher, about 40%. Furthermore, the standard curve for prednisolone which was measured with the Amerlite Cortisol assay was totally parallel with the standard curve for cortisol. The prednisolone concentrations reported here were measured as cortisol equivalents (prednisolone/cortisol) in this assay. Consequently, the concentrations reported underestimate the true prednisolone concentrations.

Statistics

Values are presented as median and range. The Wilcoxon signed rank test was used in the statistical evaluation of the results. Unless otherwhise mentioned, all comparisons were made with the control occasion before the experiment. Levels of significance are expressed

conventionally: ns = not significant, * = $p<0.05$, ** = $p<0.01$.

Results

The in vivo experiment

Cortisol/prednisolone in serum. There was a significant elevation of cortisol/prednisolone in serum after one week of treatment. The elevation was significant at time points T1-T5 (Table 1).

The WBC and differential count. The WBC was unaltered during full dose treatment but after diminishing the dose, there was a significantly lower total WBC both in the first ($p = 0.01$) and second ($p = 0.03$) week compared with the control sample. The neutrophils showed a significant elevation at time point T1 and a significantly lower number at time point T4 compared with the control sample (Fig. 1A). The lymphocyte count was significantly lower at time point T5 than in the control sample (Fig. 1B). The eosinophil count was significantly lowered during the whole treatment period (Fig. 1A), while the monocyte count was higher at T2 compared with C1 and lower at T4 than at C1 (Fig. 1B).

Other blood parameters. The hemoglobin content was significantly decreased during the treatment and the 2 control tests after cessation of the treatment, with the exception of time point T5 (Table 2). The serum concentration of alkaline phosphatase was elevated at time points T2-T5 (Table 2).

Adhesion. After treatment with corticosteroids for 3 weeks (T3), adherence of neutrophils was significantly depressed. This effect remained at time point T4 (Fig. 2).

Random migration and chemotaxis. Random migration of neutrophils was not affected by corticosteroids, while the chemotactic response to casein was significantly enhanced at time point T4, and so was the chemotactic response to zymosan activated serum at time points T2, T4 and T5 (Fig. 3).

Table 1. Cortisol/prednisolone (nmol/l) in serum before treatment (C1), after 1-3 weeks treatment (T1-3) and 2 weeks of diminishing the dose (T4-5), and 1 week after completed treatment (C2). The p-values are based on the comparison of cortisol/prednisolone in serum between the control sampling before treatment and the other samples. N = 6.

	Median (nmol/l)	Range	p-value
C1	49.0	27- 71	
T1 (1mg/kg)	278.5	185-321	0.03
T2 (1mg/kg)	143.5	72-208	0.03
T3 (1mg/kg)	238.5	190-290	0.03
T4 (0.7mg/kg)	149.0	51-355	0.05
T5 (0.3mg/kg)	91.0	10-158	0.05
C2	57.5	42- 82	ns

Phagocytosis. The number of neutrophils activated by IgG-opsonized yeast cells was enhanced from time point T2 through T4 and was decreased at C2 compared with C1 ($p<0.05$) (Fig. 4A). Ingestion of IgG-opsonized yeast cells was enhanced from time point T1 through T4 and decreased at C2 compared with C1 ($p<0.05$) (Fig. 4A). The number of activated neutrophils and ingested yeast cells was significantly decreased at T5, C2 and C3 compared with T3 ($p<0.05$) The number of neutrophils activated by serum opsonized yeast cells was unaltered during the treatment period compared with C1 (Fig. 4B). The number of activated neutrophils was decreased at T5 compared with T3 ($p<0.05$). Ingestion of serum-opsonized yeast cells was diminished at time point C2, but was unchanged compared to C1 during the rest of the period. The number of ingested yeast cells was decreased at T5, C2 and C3 compared with T3 ($p<0.05$) .

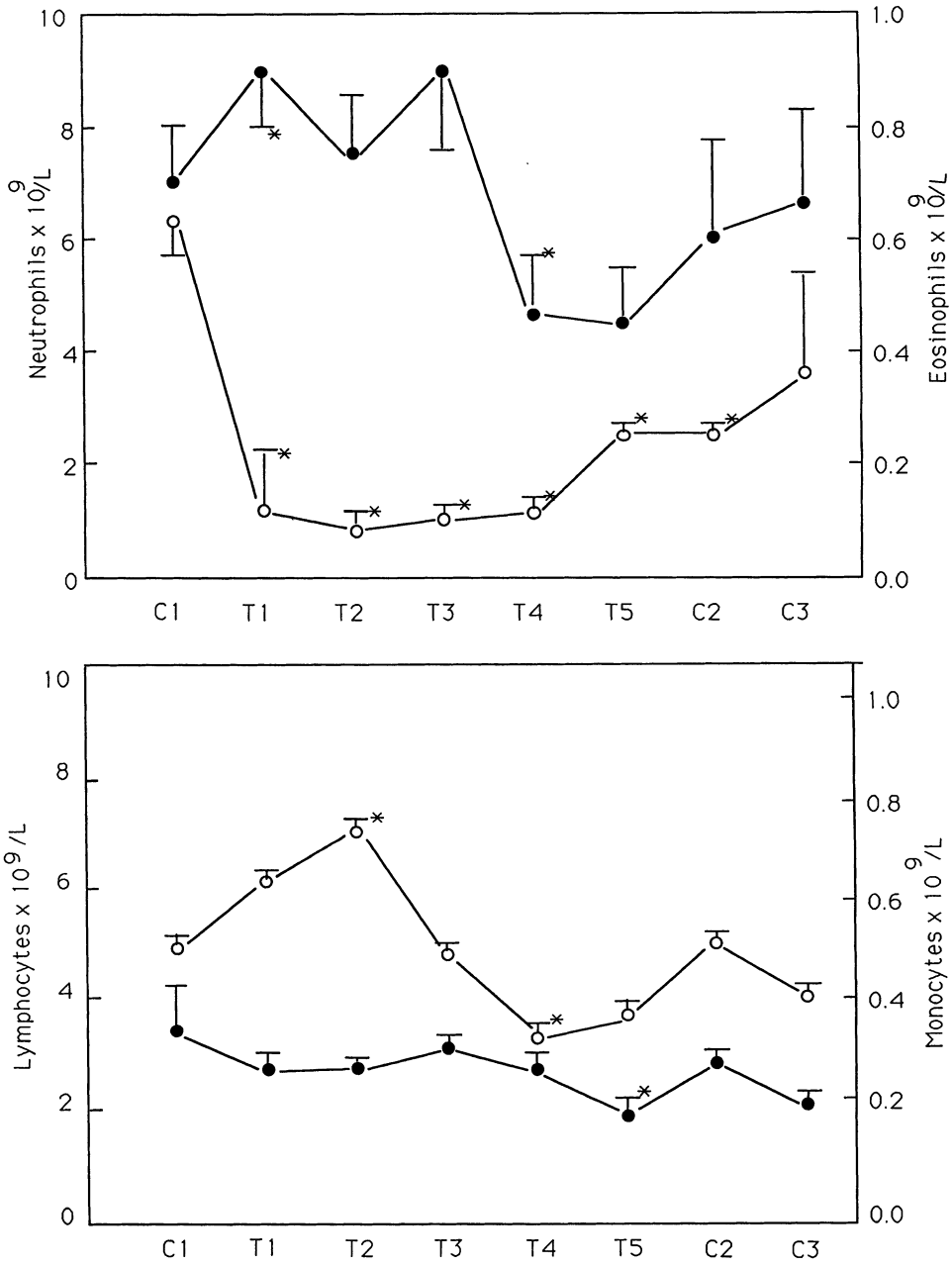

Figure 1 A. The number of neutrophils (●) and eosinophils (○) at the different time points (C1 = control before treatment, C2 and C3 = control 1 and 2 weeks after treatment period and T1-T5 = 1-5 weeks of experiment) in the in vivo administration experiment. B. The number of lymphocytes (●) and monocytes (○) at the different time points. Values are given as mean ± s.e.m., n = 8.

Table 2. The hemoglobin (Hb) content (g/L) and serum concentration of alkaline phosphatase (ALP) before treatment with prednisolone (C1) and after 1-3 weeks (T1-3) treatment (1mg/kg), after diminishing the dose (T4-5) (0.7 and 0.3 mg/kg respectively), and 1 week and 2 weeks after completed treatment (C2 and C3), n = 6.

	Hemoglobin (g/L)			Alkaline phosphatase (μkat/L)		
	Median	Range	p-value	Median	Range	p-value
C 1	155	147-159		2.0	1.6- 5.6	
T.1	143	135-164	0.05	2.7	3.8-12.4	ns
T.2	145	136-155	0.05	4.4	3.3- 8.1	0.03
T 3	135	127-141	0.03	4.5	2.4- 6.5	0.03
T.4	147	140-150	0.03	3.9	2.0- 7.3	0.03
T.5	148	143-153	ns	4.3	1.6-13.1	0.05
C 2	148	137-151	0.03	3.4	1.1- 4.5	ns
C 3	143	141-152	0.03	2.3	1- 3.7	ns

Chemiluminescence. Zymosan-induced chemiluminescence of neutrophils was elevated after 3 and 4 weeks of treatment (T3 and T4) ($p<0.05$) and returned to normal after the dose had been lowered to 0.3 mg/kg (T5) (Fig. 5).

Expression of FcγRIII, CD11b and CD18 measured by flow cytometry. No difference could be detected between the control dog and the exposed dogs, and exposed dogs showed no difference in expression of any of the receptors studied, before, after or during the time of exposure to prednisolone (results not shown). The functional uptake of IgG-op-

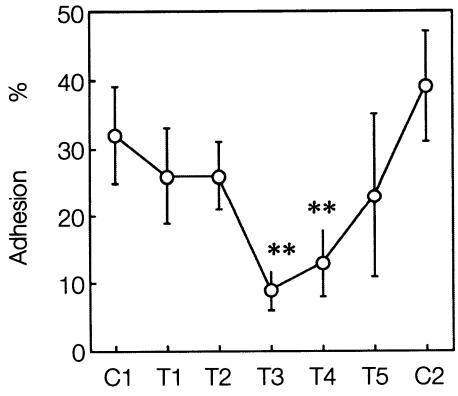

Figure 2. Adhesion of neutrophils at the different time points (C1 = control before treatment experiment, C2 = control 1 week after termination of treatment and T1-T5 = 1-5 weeks of treatment) in the in vivo administration experiment. Adhesion is expressed as the concentration of neutrophils after passage through the column in relation to the concentration before passage in %. Values are given as mean ± s. e. m., n = 8.

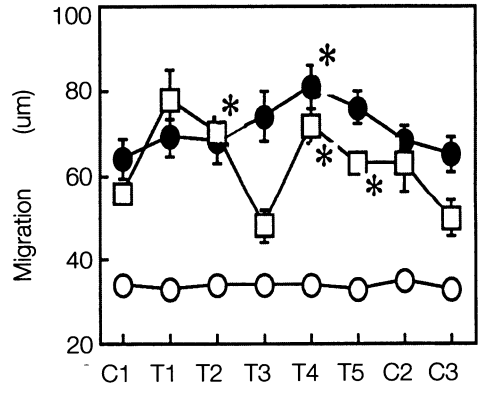

Figure 3. The distances of random migration (○) and chemotaxis with casein (●) and zymosan activated serum (□) as chemoattractants at the different time points (C1 = control before treatment experiment, C2 and C3 = control 1 and 2 weeks after cessation of treatment and T1-T5 = 1-5 weeks of treatment) in the in vivo administration experiment. Values are given as mean ± s.e.m., n = 6.

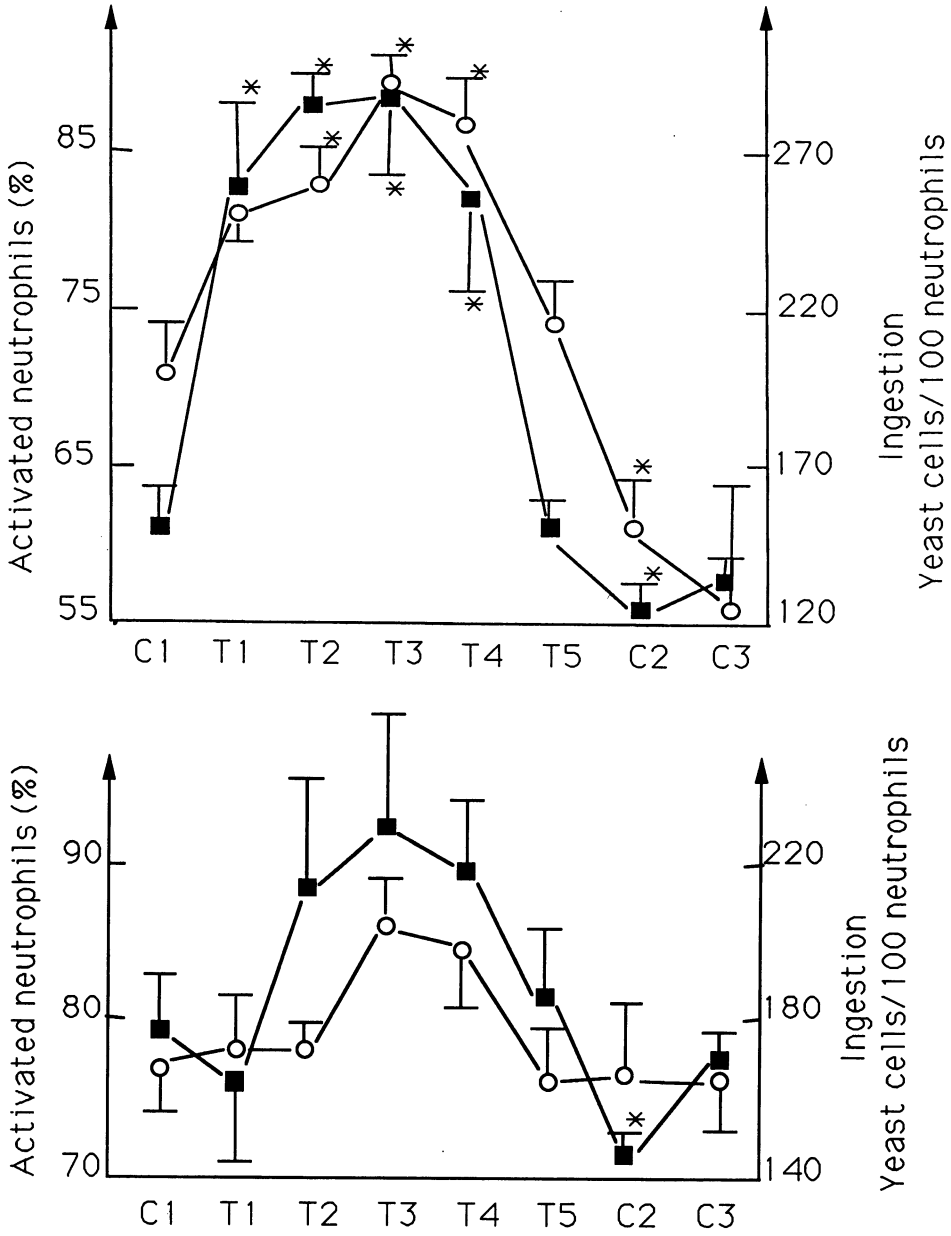

Figure 4. A. The number of activated neutrophils (○) and ingested yeast cells/100 neutrophils (■) at the different time points (C1 = control before treatment experiment, C2 and C3 = control 1 and 2 weeks after termination of treatment and T1-T5 = 1-5 weeks of treatment). Yeast cells are opsonized with anti yeast IgG.
B. The number of activated neutrophils (○) and phagocytozed yeast cells/100 neutrophils (■) at the different time points Yeast cells are opsonized with serum. Values are given as mean ± s.e.m., n = 6.

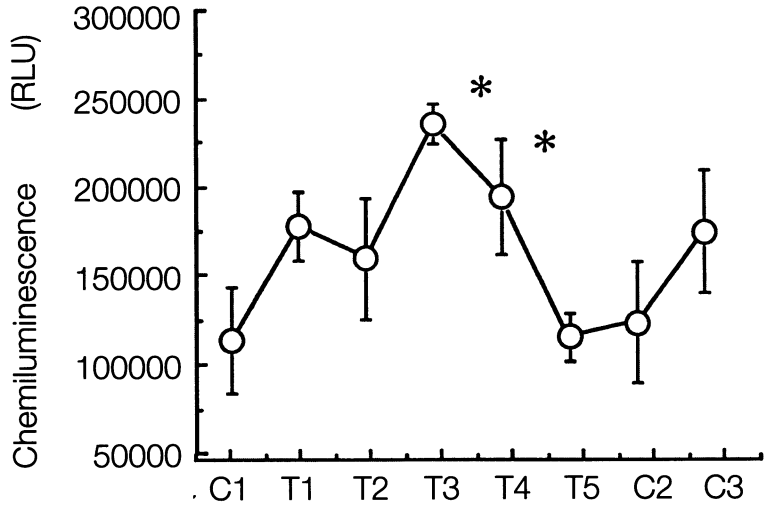

Figure 5. Zymosan induced chemiluminescence measured in Relative Light Units (RLU) at the different time points (C1 = control before treatment experiment, C2 and C3 = control 1 and 2 weeks after treatment and T1-T5 = 1-5 weeks of treatment). Values are given as mean ± s.e.m., n = 6.

sonized yeast cells increased, however, even in this experiment (results not shown).

The in vitro experiments

Adhesion. Incubation of whole blood with prednisolone resulted in a significant ($p = 0.03$) reduction of neutrophil adherence at the prednisolone concentration of 800 ng/ml in blood (results not shown).

Random migration and chemotaxis. Prednisolone did not affect random migration or chemotactic response to casein – and – zymosan activated serum of neutrophils, either when added to whole blood or to isolated neutrophils (results not shown).

Phagocytosis. Preincubation of isolated neutrophils with 50-200 ng prednisolone/ml did not affect the ingestion of IgG-opsonized yeast cells of neutrophils or the number of activated neutrophils (results not shown). Ingestion of IgG-opsonized particles by neutrophils was decreased after incubation of whole blood with 800 ng prednisolone/ml ($p = 0.03$). The number of neutrophils activated by IgG-opsonized particles was significantly diminished after incubation of whole blood with 400 ng ($p = 0.03$) and 800 ng ($p = 0.05$) prednisolone/ml. Ingestion of serum-opsonized yeast cells by neutrophils was essentially unaffected by incubation of whole blood or isolated cells with prednisolone.

Chemiluminescence. Prednisolone had no effect on zymosan-induced chemiluminescence, either when whole blood or isolated cells were used (results not shown).

Discussion

The most obvious changes in neutrophil function, induced by treatment with prednisolone in vivo, were the decreased adherence to nylon wool (Fig. 2), the increased activation and ingestion of IgG opsonized yeast cells (Fig. 4A) and the increased chemiluminescence (Fig. 5).

The chemotactic responses to complement activated serum and casein were also increased (Fig. 3). The increased chemotaxis, phagocytosis, and killing capacity might reflect an increased functional activity of neutrophils and bring about an increased clearance of IgG-complexes and microorganisms. This might be one mechanism behind the antiinflammatory effect of corticosteroids. During steroid treatment, the blood eosinophil count decreased (Fig. 1A) and the serum concentration of alkaline phophatase was elevated (Table 2). When prednisolone was added to whole blood, neutrophil adhesion and phagocytosis of IgG opsonized yeast particles were unaffected at all concentrations, except for the highest, and neutrophil migration and chemiluminescence were completely unaffected.

The elevated alkaline phosphatase (*Feldman* 1987) and the eosinopenia (*Cupps & Fauci* 1982) are well documented corticosteroid effects. Treatment with corticosteroids causes neutrophilia (*Cupps & Fauci* 1982, *Guelfi et al.* 1985, *Molina et al.* 1991) through stimulation of granulocyte colony stimulating factor and through delaying apoptosis (*Liles et al.* 1995). The fact that the increased functional responsiveness was more obvious after 2 weeks of treatment than after one week, suggests that the changes are due to effects on proliferating and differentiating neutrophils in the bone marrow, and not a direct effect on the cells in the blood. This idea is also consistent with the lack of effect in the in vitro experiments. Accordingly, the new cells produced during the treatment period would be responsible for the reported enhanced functional responses.

A decreased adherence of neutrophils to endothelium is most probably an important part of the antiinflammatory effect of corticosteroids, mediated through the neutrophils. In the present study, corticosteroid treatment in vivo decreased the adherence of neutrophils to nylon wool, but in the in vitro experiments, neutrophil adhesion was not diminished until supra pharmacological concentrations were reached. The negative in vivo effect of corticosteroids on neutrophil adhesion to nylon wool was most probably not caused by influence on the β2-integrin receptor CD11b/CD18, since the expression of the receptor was unchanged, and the phagocytosis of C3b-coated particles, which is mediated by the same receptor, was not decreased. In previous in vivo studies using supra pharmacological concentrations of corticosteroids (40 mg/kg respectively 60 mg/kg), decreased neutrophil adherence was induced after 4 h (*Mac Gregor et al.* 1974, *Clark et al.* 1979). An in vitro study using 5 to 30 ug/mL hydrocortisone sodium succinate did not show any influence on neutrophil adhesion to nylon wool (*Mac Gregor et al.* 1974).

During recent years some in vivo studies on the influence of corticosteroids have shown enhanced neutrophil function at pharmacological doses, thereby supporting the results of the present study. Investigations of migratory responses showed augmented chemotactic responses of equine neutrophils after administration of hydrocortisone (1mg/kg) (*Morris et al.* 1988), and increased random migration and chemotaxis of canine neutrophils after administration of prednisolone (4mg/kg during 3 days) (*Guelfi et al.* 1985). The elevated chemotactic response might be a result of an influence of corticosteroids on the functional capacity of the receptors for C5a (*Webster et al.* 1980) and casein (*Lewis & Epps* 1983) or a general enhancement of the migratory responses induced by chemotactic factors.

The pronounced increase in the IgG-mediated phagocytosis induced by prednisolone treatment was dose dependent and also decreased significantly when the dose was lowered to 0.3 mg/kg. The cause of the increased Fc-receptor mediated phagocytosis is most probably an en-

hanced functional capacity of the receptors measured, as cell surface expression was unaltered. The C3b-dependent phagocytosis also decreased significantly when the doses declined to 0.3 mg/kg, which indicates that high therapeutic doses of prednisolone influences C3b-mediated phagocytosis.

The increase of chemiluminescence induced by serum-opsonized particles could be a result of the increased phagocytosis, but might also be due to a direct effect of prednisolone on the enzyme system responsible for the respiratory burst. One previous study showed a remarkable enhancement of the chemiluminescence of neutrophils from humans receiving methylprednisolone infusion (*Kuzniar et al.* 1991), and another study found that therapeutic doses of dexamethasone caused enhanced chemiluminescence of bovine neutrophils, while it had no effect in vitro (*Phillips et al.* 1987). In a third study, increased neutrophil ingestion and killing capacity was induced by methylprednisolone (1mg/kg) (*Molina et al.* 1991).

The many in vitro studies of the effect of corticosteroids on the neutrophil function show inhibition or no influence by high supra therapeutic doses compared with serum concentrations obtained at therapeutic doses (*Colburn et al.* 1976). Hydrocortisone at the concentration of 2.1 mM decreased intracellular killing and the ability to reduce nitro blue tetrazolium by isolated neutrophils, probably by impairment of NADPH oxidase activity (*Mandell et al.* 1970). Hydrocortisone was also found to reduce fMLP induced CR1 and CR2 receptor mobilisation and ingestion (*Forslid et al.* 1987), and methyl prednisolone at very high concentrations (1mg/mL) was shown to impair neutrophil ingestion (*Baltch et al.* 1986). In vitro studies of the impact of corticosteroids on chemiluminescence support the results of the present study, i. e. that at concentrations corresponding to those during corticosteroid therapy there is no impairment of the oxidative metabolism (*Levine et al.* 1981, *Niwa et al.* 1987). *Olds et al.* (1974) stated that high doses of methylprednisolone or hydrocortisone in vitro do not impair phagocytic bactericidal capacity and that impairment of these functions are only obtained at concentrations markedly exceeding the high therapeutic concentrations in humans.

The present study indicates that the in vivo effects of a general therapeutic corticosteroid treatment on neutrophil functions are essentially different from those obtained by in vitro treatment of whole blood or isolated cells. The inhibition of neutrophil adhesion that appeared after 2 weeks of treatment is most probably mediated by another mechanism than the direct inhibition obtained in vitro. When administered in vivo in therapeutic doses there was an enhancing effect on phagocytosis and killing capacity, which might be an important mechanism behind the anti-inflammatory action of corticosteroids in addition to the decreased adherence. In vitro corticosteroid therapy only occasionally dampened neutrophil function, otherwise it had no influence at all at doses corresponding to therapeutic serum concentrations.

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Sammandrag

In vivo- och in vitroeffekter av prednisolon på granulocytfunktioner hos hund.

I föreliggande studie undersöktes in vivo-effekten av en terapeutisk dos prednisolon på neutrofilers adhesion, migration/kemotaxis, fagocytos och chemiluminescence hos hund. Prednisolonets påverkan på antalet FcγIII-receptorn och CR3-receptorer studerades också. Prednisolon tillsattes även in vitro till neutrofilsuspension och effekten på neutrofilernas funktion studerades. Resultaten visar att det föreligger skillnader mellan effekten av prednisolon in vivo och in vitro. När prednisolon gavs in vivo, minskade neutrofilernas adhesion och den fagocyterande och avdödande förmågan ökade. Antalet Fc- och CR3-receptorer påverkades inte av behandlingen. När prednisolonet tillsattes in vitro till cellsuspensioner hade det ingen eller dämpande effekt på neutrofilerna. Resultaten tyder på att en del av den antiinflammatoriska effekten vid kortisonterapi in vivo, förutom den minskade adhesiviteten, kan vara ökad eliminering av mikroorganismer och IgG-komplex genom ökad kapacitet hos neutrofilerna.

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