

Duration of blood plasma cortisol suppression after a low-dose dexamethasone suppression test in dogs

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Abstract The aim of this study was to determine the duration of the suppressive effect on the hypothalamus–pituitary–adrenocortical axis as measured by plasma cortisol concentration after injection of a low dosage of dexamethasone (0.01 mg/kg BW) used within the low-dose dexamethasone suppression test (LDDST). A LDDST was performed on ten clinically healthy dogs with unremarkable haematological and plasma biochemical profiles, abdominal X-ray, abdominal ultrasonography and fine needle aspiration cytology of the liver. Two of them were excluded from the analysis. Blood samples were collected at baseline, 4 and 8 h (standard protocol of a LDDST) and, additionally, at 24, 48, 72 and 96 h after the application of dexamethasone. Plasma cortisol concentrations <10 ng/ml were considered as suppressed. All eight dogs had suppressed plasma cortisol levels after 4 and 8 h. Three dogs still had suppressed plasma cortisol levels at 24 h after dexamethasone application, and one of them even had a suppressed level after 48 h. After 72 h, all dogs had plasma cortisol levels >10 ng/ml. The median 4, 8 and 24 h plasma cortisol concentrations were significantly lower than the median baseline plasma cortisol concentration, whereas at the remaining times no significant changes were seen compared to the median baseline value. The suppression of plasma cortisol induced by dexamethasone in a dosage of 0.01 mg/kg BW can last at least 48 h in individual dogs. Therefore, a

repeated LDDST should be performed at the earliest 3 days after the prior test to prevent artificial results.

Keywords Pituitary–adrenocortical axis · Plasma cortisol · Cushing syndrome · Hyperadrenocorticism · Canine

Introduction

The low-dose dexamethasone suppression test (LDDST) is the test of choice to diagnose hyperadrenocorticism in suspicious dogs in addition to clinical and other findings. Due to a negative feedback in healthy dogs, the application of dexamethasone inhibits the secretion of ACTH by the pituitary and therefore it suppresses the endogenous blood plasma cortisol level. In dogs with hyperadrenocorticism, this negative feedback becomes more or less ineffective because of the autonomous production of ACTH or cortisol, respectively, so that an administration of dexamethasone in a low dosage has no suppressive effect on the plasma cortisol concentration. To diagnose hyperadrenocorticism, the 8 h plasma cortisol value after the application of dexamethasone in a dosage of 0.01 mg/kg BW is determining (Gilor and Graves 2011; Behrend et al. 2013). There is limited information available regarding the duration of the suppressive effect of such a low dosage of dexamethasone on the dog's plasma cortisol level, which is a useful information, for example, when it is necessary to repeat the test due to an inconclusive result of the initial test. There are two studies that describe the duration of the plasma cortisol suppression after dexamethasone injection in dogs. Kempainen and Sartin (1984) tested the duration of plasma cortisol suppression after intravenous injection of 0.01 mg/kg BW of dexamethasone or dexamethasone sodium phosphate in eight dogs and the tenfold dosage in another eight dogs (compared with a control group of four dogs), but

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they only presented the mean and standard error of the mean (SEM) and not the plasma cortisol values of individual dogs. The authors concluded from their results that mean plasma cortisol concentration is suppressed for less than 24 h for the lower dosage. Toutain et al. (1983) studied only higher dosages of dexamethasone so that the results of that study cannot be transferred to studies where information regarding the duration of plasma cortisol suppression after a LDDST is needed. Therefore, the aim of this study was to determine the duration of suppression of plasma cortisol concentration after application of dexamethasone in the standard dosage used in LDDST in individual healthy dogs.

Materials and methods

Study design

A LDDST with a dexamethasone dosage of 0.01 mg/kg BW was performed in ten healthy beagle dogs. After taking a blood sample for baseline (0 h) plasma cortisol measurement, dexamethasone was injected intravenously and further blood samples were taken 4 and 8 h later following the standard protocol of a LDDST. Additional blood samples were taken at times 24, 48, 72 and 96 h after dexamethasone application, and plasma cortisol was measured. The interpretation of plasma cortisol levels was based on the reference ranges of cortisol of the Endocrinology Laboratory of the University of Veterinary Medicine Hannover, Foundation (15–35 ng/ml). After dexamethasone application, plasma cortisol concentrations <10 ng/ml were considered as suppressed.

Animals

All healthy dogs were beagles. Five dogs were recruited by the Department of Pharmacology, Toxicology and Pharmacy of the University of Veterinary Medicine Hannover, Foundation and five dogs were owned by the Institute for Parasitology of the University. Approval of German legislation of animal welfare was given (No. 13/1247). The median age was 5.8 (range 2.6–6.4) years. The group of dogs consisted of one intact male, two neutered males, four intact females and three spayed females. All dogs underwent a clinical examination without abnormal findings and were declared clinically healthy. In addition, they had physiological haematological and plasma biochemical profiles. The dogs got x-rayed with explicit inspection of liver size, which was normal in all dogs. An ultrasonography of the abdomen was performed, and all dogs had adrenal glands smaller than 7.5 mm which were considered as normal. A fine needle aspiration of the liver was performed in each dog, and the cytological cell image occurred to be unremarkable. None of the dogs was on any medication, and they did not receive any

glucocorticoids within the last 6 months. For the whole test period, the dogs stayed in their familiar environment in the above-cited institutes. They were kept in groups of two or three dogs in a tempered room with access to an outside accommodation. Two of the ten dogs were excluded from the analysis because of plasma cortisol levels which were not compatible with results of healthy dogs. One dog had a positive result in the LDDST but the control LDDST 11 weeks later was negative. The second dog had a subnormal blood plasma cortisol level during the whole test period including the baseline plasma cortisol level before dexamethasone was applied.

Sample collection and sample preparation

Dexamethasone application Between 09.00 and 10.00 a.m., a 22-gauge permanent venous catheter was inserted in the cephalic or lateral saphenous vein through which 0.01 mg/kg BW of dexamethasone as dexamethasone dihydrogen phosphate disodium (CP-Pharma, Burgdorf, Germany) was injected intravenously. To dose as precise as possible, the commercial dexamethasone solution (2 mg/ml) was diluted 1:10 with isotonic sodium chloride solution to achieve a concentration of 0.2 mg/ml. The catheter was removed after the injection.

Blood collection For the measurement of the baseline plasma cortisol value, blood was taken through the recently inserted permanent venous catheter before dexamethasone was injected. At each of the remaining above-cited time points (see [Study design](#)), blood samples were taken by venipuncture with a 22-gauge needle. At each time point, 1.3 ml blood was collected into a lithium-heparin-containing tube (for plasma cortisol measurements) and two further tubes were in addition filled with blood for another study (a further 1.3 ml lithium-heparin-containing tube and a 1.1 ml-serum tube).

Sample preparation After centrifugation at about 17,000×g for 2 min at room temperature, plasma was separated and stored at –80 °C until hormone analysis was performed.

Cortisol measurement

Plasma cortisol was determined using the Immulite System (Siemens Healthcare Diagnostics, Eschborn, Germany) which is a competitive solid-phase chemiluminescence immunoassay. For this cortisol test kit, intraassay coefficients of variation (CVs) of 5.4–8.8 % and interassay CVs of 6.3–10.0 % were assessed.

Statistics

To assess standard normal distribution of data, we used the Kolmogorov-Smirnov test. Because data were not standard normally distributed at all times, plasma cortisol values at

different time points were summarized to median (minimum–maximum) with the use of Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Additionally, calculated relative (%) values (compared to baseline) were illustrated by box and whisker plots. Consequently, non-parametric tests (Friedman and Wilcoxon test for repeated measures) were used to compare plasma cortisol concentrations of different times and of individual times to baseline. A p value <0.05 was considered statistically significant, i.e. Bonferroni correction was not considered. Statistical analyses were performed by the use of IBM SPSS Statistics 22 (Chicago, IL, USA).

Results

At times 4 and 8 h, the plasma cortisol concentrations of all eight dogs were below the cutoff value of 10 ng/ml. Twenty-four hours after the injection of dexamethasone, plasma cortisol levels of three dogs were still below the cutoff and the plasma cortisol concentration was still suppressed even after 48 h in one dog (Table 1). Apart from median 4 and 8 h values, also the median 24 h value was significantly lower than the median baseline value ($p<0.05$, Wilcoxon test; $p=0.000$, Friedman test). However, after 24 h, only a slight inhibition (median of relative values compared to baseline, 69 %) was seen when compared to 4 and 8 h values (15 or 18 %, respectively) (Fig. 1).

Discussion

The results of the study demonstrate that a significant, although reduced, suppressive effect of the tested dexamethasone dosage was still present after 24 h. Thereby,

our results differ slightly from the results reported by Kemppainen and Sartin (1984) who showed significant differences at 16 h but not at 24 h after a single intravenous dexamethasone or dexamethasone sodium phosphate injection, respectively, at a dosage of 0.01 mg/kg BW to groups of four healthy dogs. One possible explanation for this slight difference could be that statistical comparison in the cited study was performed to a control group of four dogs receiving saline instead of dexamethasone rather than to baseline values of the treatment group as in the present study. However, graphs which illustrate means and SEMs indicate that mean cortisol levels after 24 h were nearly identical with baseline values, suggesting that the discrepant reference for statistical comparison is not the reason for the discrepancy regarding this study. In contrast, statistical analysis has some relevance for the interpretation of data in a further study (Bugbee et al. 2013). In that study, endogenous ACTH was measured in healthy dogs after intravenous injection of 0.01 mg/kg dexamethasone and was significantly decreased when compared to a control group receiving a saline injection at 8 and 12 h after the injection, but not 24 h after the injection. However, when comparing the graphically presented mean values, it becomes clear that ACTH blood concentrations in the treatment group 24 h after the dexamethasone injection were still approx. one third lower when compared to baseline concentrations which corresponds well to the persistently reduced cortisol values at this time period in our study.

Results of the present study and of the study of Kemppainen and Sartin (1984) indicate remarkable inter-individual variation in sensitivity of endogenous cortisol towards dexamethasone. Thus, it is not surprising that results based on small groups can differ. As only means and SEMs are illustrated, it is possible that

Table 1 Plasma cortisol values (ng/ml) of healthy dogs at different time points after intravenous application of 0.01 mg/kg dexamethasone

Dog	Sex	Age (years)	Time (h after dexamethasone)						
			0	4	8	24	48	72	96
1	Female, intact	3.8	34.3	2.8	7.2	26.8	27.9	23.7	36.5
2	Male, neutered	6.1	27.2	4.4	5.0	9.8	8.2	18.2	16.4
3	Male, neutered	6.1	20.3	2.8	2.5	4.3	18.5	18.8	18.4
4	Female, spayed	6.1	18.4	9.8	9.0	20.7	19.1	36.5	23.7
5	Female, spayed	6.4	24.9	4.4	3.7	16.2	27.4	22.8	18.3
6	Male, intact	5.5	43.6	2.6	7.7	31.2	24.4	34.1	19.5
7	Female, intact	3.5	11.3	2.6	8.1	11.8	19.6	12.6	15.6
8	Female, intact	2.6	21.0	2.7	2.1	5.3	12.1	19.0	11.1
	Median	5.8	23.0	2.8	6.1	14.0	19.4	20.9	18.4
	Minimum	2.6	11.3	2.6	2.1	4.3	8.2	12.6	11.1
	Maximum	6.4	43.6	9.8	9.0	31.2	27.9	36.5	36.5

also in the study of Kemppainen and Sartin (1984), individual dogs had a longer suppression. In our study, in one individual dog, a residual suppressive effect was still detectable after 48 h. Based on mean values of groups of experimental dogs, suppression of the adrenal gland function for 24 h or longer has been reported after higher dosages of dexamethasone. In the study of Kemppainen and Sartin (1984) one group receiving 0.1 mg/kg of dexamethasone had reduced values after 32 h, whereas results after 40 h were not different from controls. A further tenfold increase in dexamethasone dosage (1 mg/kg) was associated with a suppression lasting for at least 24, but less than 48 h, when the results were similar to baseline values (Toutain et al. 1983). The partly longer-lasting suppression in individual dogs even after 0.01 mg/kg BW seems to be new information which indicates that in the case that a repeated LDDST is necessary, it should be performed at the earliest after 3 days.

Reasons for the considerable inter-individual variation regarding the reaction of the hypothalamic–pituitary–adrenal (HPA) axis may be variations in the binding proteins transcortin and albumin, which bind more than 90 % of the cortisol in the circulation (Bansal et al. 2015). Therefore, alterations in these proteins can significantly influence the measured cortisol concentration and interpretation of stimulation or suppression tests (Bansal et al. 2015). In addition, periods of activity and rest influence the HPA axis and may vary between individual dogs and different studies. In a large human study, enhanced negative feedback of the HPA axis—as

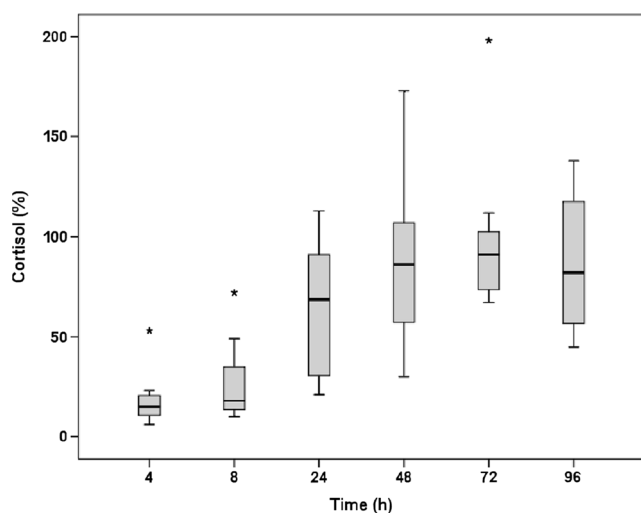


Fig. 1 Plasma cortisol values of healthy dogs at different time points after application of 0.01 mg/kg dexamethasone. Results are given as relative values (%) compared to baseline values. Illustration as *box and whisker plots* representing median, 25–75 % percentile (*box*), minimum and maximum (*whiskers*), and outliers (*asterisks*; extend >1.5 box lengths from the edge of the box)

measured as the change in morning saliva cortisol after the intake of 0.25 mg dexamethasone the night before—was found in association with unstable activity rhythms, total sleep duration, and poor subjective sleep quality (Luik et al. 2015).

In contrast to Kemppainen and Sartin (1984), our dogs underwent not only a thorough clinical examination, but also haematological and plasma biochemical profile screenings as well as X-ray, ultrasonography of the abdomen, and fine needle aspiration cytology of the liver to confirm health status and to completely exclude individuals with hyperadrenocorticism. However, although we tried carefully to exclude dogs with signs of hyperadrenocorticism by these different examinations, one beagle dog had cortisol values consistent with a positive LDDST and therefore had to be excluded from the statistical analyses. These results underline the well-known fact that diagnosis of hyperadrenocorticism should not only be based on the results of a LDDST, but should always consider a panel of parameters including clinical signs, size of the liver and adrenal glands, results of haematological and clinicochemical tests in addition to results of the LDDST (Gilor and Graves 2011; Bansal et al. 2015). Accordingly, two of 10 clinically healthy experimental dogs receiving stimulation with synthetic ACTH showed a significant increase of plasma cortisol concentration (261 and 211 ng/ml) to an area usually suggestive for hyperadrenocorticism (Bugbee et al. 2013).

A limitation of our study is the exclusive use of beagle dogs. In addition, as other authors (Toutain et al. 1983; Kemppainen and Sartin 1984), we tried to stress the dogs as little as possible by collecting the blood samples in their familiar environment. However, an impact of stress on the plasma cortisol levels cannot be completely ruled out. A further aspect which may be debatable is the variation in blood collection technique. We collected baseline cortisol samples through a recently inserted permanent venous catheter, whereas further blood samples were withdrawn through venipuncture with a 22-gauge needle. However, this should not have significantly influenced results: no significant differences in plasma cortisol values were measured in blood samples collected through an indwelling intravenous catheter in the jugular vein or venipuncture although the primary aim of the study was to assess the effect of pain caused by venipuncture on plasma cortisol levels amongst other things (Knol et al. 1992).

In conclusion, our study indicates a long-lasting suppressive effect of 0.01 mg/kg BW of dexamethasone in individual dogs, so that a repeated LDDST should be performed at the earliest 3 days after the prior test to prevent artificial results.

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Statement on human and animal rights All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of interest S. Hoffrogge has received research grants from the Bruns-Stiftung.

M. Schmicke declares that she has no conflict of interest.

R. Mischke declares that he has no conflict of interest.

Statement of informed consent Informed consent was obtained from all individual participants included in the study.

References

- Bansal V, Asmar NE, Selman WR, Arafah BM (2015) Pitfalls in the diagnosis and management of Cushing's syndrome. *Neurosurg Focus* 38(2):E4. doi:10.3171/2014.11.FOCUS14704
- Behrend EN, Kooistra HS, Nelson R, Reusch CE, Scott-Moncrieff JC (2013) Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (small animal). *J Vet Intern Med* 27(6):1292–1304. doi:10.1111/jvim.12192
- Bugbee AC, Smith JR, Ward CR (2013) Effect of dexamethasone or synthetic ACTH administration on endogenous ACTH concentrations in healthy dogs. *Am J Vet Res* 74(11):1415–1420. doi:10.2460/ajvr.74.11.1415
- Gilor C, Graves TK (2011) Interpretation of laboratory tests for canine Cushing's syndrome. *Top Companion Anim Med* 26(2):98–108. doi:10.1053/j.tcam.2011.03.001
- Kemppainen RJ, Sartin JL (1984) Effects of single intravenous doses of dexamethasone on baseline plasma cortisol concentrations and responses to synthetic ACTH in healthy dogs. *Am J Vet Res* 45(4):742–746
- Knol BW, Dieleman SJ, Bevers MM, Van den Brom WE, Molt JA (1992) Effects of methods used for blood collection on plasma concentrations of luteinising hormone, testosterone, and cortisol in male dogs. *Vet Q* 14(4):126–129
- Luik AI, Direk N, Zuurbier LA, Hofman A, Van Someren EJ, Tiemeier H (2015) Sleep and 24-h activity rhythms in relation to cortisol change after a very low-dose of dexamethasone. *Psychoneuroendocrinology* 53C:207–216. doi:10.1016/j.psyneuen.2015.01.011
- Toutain PL, Alvinerie M, Ruckebusch Y (1983) Pharmacokinetics of dexamethasone and its effect on adrenal gland function in the dog. *Am J Vet Res* 44(2):212–217