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Adrenocortical Suppression by a Glucocorticoid: Effect of a Single i. m. Injection of Betamethasone Depot Versus Placebo given Prior to Orthopaedic Surgery in Dogs

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Mbugua, S. W., L. A. Skoglund, O. Djøseland and P. Løkken: Adrenocortical suppression by a glucocorticoid: Effect of a single i. m. injection of betamethasone depot versus placebo given prior to orthopaedic surgery in dogs. Acta vet. scand. 1988, 29, 415–419. – The suppression of the endogenous serum cortisol level by a single i. m. injection of 3 mg betamethasone depot (Celeston Chronodose®) prior to orthopaedic surgical interventions on the forelimbs of 8 dogs was studied in a placebo-controlled cross-over trial. Compared to placebo the endogenous serum cortisol level was significantly ($p < 0.05$) reduced for a period of 4 days, and then returned to the pre-operative range. During the 28-day observation period after each surgical intervention no impairment of wound healing, bleeding episodes or side-effects which could be attributed to the glucocorticoid, was seen.

cortisol suppression; wound healing.

Introduction

Exogenous administration of glucocorticoids is used in the veterinary as well as in the human clinic to alleviate various inflammatory conditions (*Austin* 1979).

The production of natural glucocorticoid hormones such as cortisol is controlled by endogenous production of adrenocorticotropic hormone (ACTH) which in turn is influenced by a negative feedback mechanism controlled by the endogenous cortisol level. This control mechanism is referred to as the hypothalamico-pituitary-adrenal (HPA) axis and may be depressed by the interference of exogenous glucocorticoid administration which after prolonged administration may lead to the induction of pathological changes in the organism (*Austin* 1979).

The effect of i. m. 3 mg betamethasone depot formulation on post-operative swelling, pain and loss of function has previously been investigated in dogs following experimental orthopaedic surgery on the forelimbs (*Mbugua et al.* 1988). The aim of the present study was to investigate the effect of betamethasone depot on the endogenous cortisol level in dogs subjected to surgical interventions on the forelimbs. Dogs were injected prior to surgery and evaluated for any clinically apparent adverse effects.

Material and methods

Experimental design

The trial was a randomized, placebo-controlled crossover study where the animals acted as their own controls. Two "identical" surgical soft tissue/bone interventions were made on the forelimbs of each animal with an interval of 28 days to allow a paired comparison of the postoperative courses. A further observation period of 28 days after the last operation was utilized to allow a comparative observation of wound healing after both operations.

Animals

Eight mongrel dogs, weighing from 15 to 20 kg, of either sex and without verifiable diseases were used. Prior to the study ethical approval was obtained from the relevant authority at the Faculty of Veterinary Medicine, University of Nairobi.

Drugs

Immediately before each operation a single dose of either 3 mg betamethasone (0.5 ml celeston Chronodose®, Schering Corp., USA) or placebo (0.5 ml saline) was injected into the upper thigh muscle. The random system ensured that one half of the group received betamethasone before the first operation and the other half before the second operation. To keep the medication regimes blind to the surgeon the injections were administered by an assistant who had no other responsibility in the trial.

Surgical procedure

The operations were performed under general thiopentone/halothane anaesthesia after premedication with acetylpromazine and atropine. Halothane was chosen as the anaesthetic as variations in the halothane concentration do not affect the endogenous cortisol response caused by the surgery (*Lacoumenta et al.* 1986). During irrigation with an aqueous solution of crystalline benzylpenicillin (Penicillin G, Dawa Pharmaceuticals Ltd., Nairobi, Kenya), a skin incision was made dorsally over the third metacarpal bone of the forelimb. The metacarpus was then transected at about the midpoint with an oscillating saw (AMSCO Hall Surgical, Santa Barbara, USA). The transection was stabilized with a 6 hole mini Dynamic Compression Plate (Synthes®, Waldenburg, Switzerland), and the wound was sutured.

Blood sampling and determination of cortisol

Venous blood was drawn from each dog into a capped glass tube between 8.30 and 9.00 (am) and/or between 3.00 and 3.30 (pm). The blood samplings started pm on the 4th preoperative day to acclimatize the dogs to the procedure. Preoperative blood samples were taken day 4 pm, day 3 am and pm, day 2 am and pm, and day 1 am only. All operations were carried out before noon and a postoperative blood sample was taken (pm) the same day. Remaining postoperative blood samples were taken day 1 am and pm, day 2 am and pm, day 3 am, day 4 am and day 5 am. The blood was allowed to clot for at least 1/2 h before separation of the serum which was then stored at – 20°C.

Serum cortisol was assayed using Immunochem's Covalent-Coat Radioimmunoassay kit (Immunochem Corp., Carson, CA, USA.

Wound healing and side-effects

Clinical side-effects, bleeding episodes or any impairments of the wound healing which could be related to the medication, were assessed daily throughout the 28-day observation period.

Statistical analysis

Technical difficulties resulted in loss of

some of the serum volumes. Because of the matched-pair data analysis, both samples were withdrawn from the analysis if one of the matching serum pairs was lost. Hence, the results presented are based on the analysis of 4 to 8 matching pairs of cortisol analyses per time point.

Analysis of the data was made with a two-sided Wilcoxon matched-pairs signed-ranks test with correction for ties. A significance level of 5 % was used.

Results

The median time courses of the endogenous serum cortisol level in 4 to 8 dogs after treatment with either 3 mg betamethasone or placebo before orthopaedic surgical interventions are presented in Table 1. The cortisol levels were significantly depressed after the injection of betamethasone depot compared to placebo, from the after-noon on the day of surgery until the morning of the fifth post-operative day. The observed elevation of the median endogenous cortisol level from the first blood collection at the fourth pre-operative day to the first pre-operative day was not statistically significant. Neither was the elevation of the endogenous cortisol level from the first pre-operative day to the day of operation when receiving placebo (Table 1).

Daily clinical assessments of wound healing revealed neither abnormalities in the wound healing nor bleeding episodes during any of the post-operative observation periods. No side-effects which could be attributed to the

Table 1. Median, range and mean values of endogenous serum cortisol levels determined by radioimmunoassay in 4–8 dogs treated with a single i. m. injection of 3 mg betamethasone depot or placebo on the morning prior to "identical" orthopaedic surgical intervention. Median values were tested with Wilcoxon matched-pairs signed-ranks test with correction for ties.

Day	No. of animals	3 mg betamethasone (nmol/l)			Placebo (nmol/l)			p level
		Median	Range	Mean	Median	Range	Mean	
Pre 4 pm	6	28.5	23.0–53.0	32.3	32.0	13.0–59.0	34.8	N.S.
Pre 3 am	7	32.0	15.0–49.0	33.6	35.0	13.0–42.0	33.4	N.S.
Pre 3 pm	8	33.5	14.0–67.0	35.1	26.0	13.0–60.0	31.4	N.S.
Pre 2 am	6	35.0	23.0–74.0	40.0	32.5	14.0–87.0	38.7	N.S.
Pre 2 pm	8	42.5	24.0–63.0	43.5	35.0	14.0–67.0	39.6	N.S.
Pre 1 am	8	48.0	24.0–68.0	45.5	43.0	24.0–60.0	41.8	N.S.
Op pm	5	13.0	1.0–24.0	11.4	60.0	33.0–76.0	59.4	0.043
Post 1 am	5	9.0	0.1–12.0	8.2	48.0	28.0–66.0	48.8	0.043
Post 1 pm	6	6.5	3.0–14.0	6.8	38.5	15.0–52.0	36.8	0.027
Post 2 am	8	9.0	3.0–24.0	9.4	44.5	13.0–72.0	42.6	0.012
Post 2 pm	8	6.0	2.0–31.0	8.4	40.5	17.0–56.0	37.5	0.012
Post 3 am	5	6.0	3.0–24.0	10.0	28.0	24.0–32.0	27.6	0.043
Post 4 am	7	26.0	4.0–45.0	24.7	30.0	13.0–82.0	46.9	0.018
Post 5 am	4	29.5	23.0–41.0	30.8	43.0	19.0–71.0	44.0	N.S.

Pre = preoperative day, Op = day of operation.
Post = postoperative day.
am, pm = samples obtained between 8.30 and 9.00 a.m., and between 3.00 and 3.30 p.m., respectively.
N.S. = not significant (significance level set to $p = 0.05$).

test medication, were encountered during the trial.

Discussion

In the present study, a single i. m. injection of 3 mg betamethasone depot suppressed the endogenous cortisol level for a period of four days when injected in the morning before the surgery. Single oral administration of 0.1 mg/kg dexamethasone to dogs has been reported to suppress the endogenous cortisol level for 32 h (*Johnston & Mather* 1978). The longer lasting effect of the presently used preparation can be explained by its formulation as a combination of the easily soluble, rapidly acting disodium phosphate, and the slow dissolving acetate (*Valtonen* 1978).

Cortisol levels in dogs do not show the circadian variations (*Gordon & Lavie* 1985) demonstrated in other species (*Conroy & Mills* 1970, *Katz et al.* 1975, *Moore-Ede et al.* 1977). In dogs there is episodic secretion of endogenous cortisol with about 10 secretory episodes during a 24 h period (*Kemppainen & Sartin* 1984). No significant difference between any median cortisol value determined from blood samples taken am or pm on corresponding days during the pre-operative training period, was found in the present study. This observation supports the reports of no circadian cortisol secretion in dogs.

The observed median cortisol level in the present study showed a non-significant rise during the pre-operative training period up to the day prior to operation. The repeated placements of a syringe into a superficial vein to obtain blood samples and fluid replacement, increasingly constitute a stress which may alter the pituitary-adrenocortical activity (*Kemppainen & Sartin* 1984).

The median peak cortisol level after placebo, seen pm on the day of the operation may reflect stress-experience during the surgical intervention. It has been reported that even small haemorrhage significantly increase the endogenous cortisol secretion rate and the systemic cortisol concentration (*Dempsher & Gann* 1983). The difference between the measured cortisol level after the operation and the pre-trial level on the first pre-operative day was, however, not significant. The lack of a significant difference may depend on the relatively small number of valid serum cortisol data included in the study and interindividual data variation.

A noticeable observation was that stress associated with the surgical intervention only caused a moderate increase of the serum cortisol level. Actually, when placebo was injected, the median level remained within the baseline range reported to be normal in the dog (*Johnston & Mather* 1978, *Takahashi et al.* 1981).

Exogenous glucocorticoid administration in too large or prolonged doses may seriously interfere with the endogenous glucocorticoid level (*Austin* 1979). The present study demonstrated that a single i. m. injection of betamethasone (Celeston Chronodose®) only caused a transient lowering of the endogenous serum cortisol level, associated with no clinically apparent side effects or disturbances in wound healing.

In conclusion, it can be stated that single administration of a depot formulation of betamethasone prior to orthopaedic surgical interventions is essentially safe and does not cause excessive prolonged endogenous cortisol suppression in dogs.

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Sammendrag

Adrenocortical suppresjon av et glucocorticoid: Effekt av en i. m. injeksjon av betamethason depot sammenlignet med placebo gitt før kirurgiske inngrep hos hunder.

3 mg betamethasons (Celeston Chronodose®) evne til å undertrykke det endogene serum cortisol speil ble undersøkt etter engangs administrasjon før ortopedisk kirurgiske inngrep på forpotene hos 8 hunder i en placebo kontrollert, dobbelt blind overkryssningsstudie. 3 mg betamethason gav en statistisk signifikant ($P < 0.05$) undertrykking av det endogene serum cortisol nivå for en periode av 4 dager etter administrering. I løpet av den 28 dager lange observasjonsperioden etter hvert kirurgisk inngrep ble det ikke registrert noen forstyrrelse av sårheling, blødningsepisoder etter bivirkninger som kunne tilbakeføres til forsøkspreparatet. Engangs administrering av en depotformulering med 3 mg betamethason til hunder før ortopedisk kirurgiske inngrep gir en forbigående undertrykking av det endogene serum cortisol nivå som ikke er forbundet med noen klinisk påvisbare forstyrrelser av den post-operative sårheling.

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