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Endocrine and Immune Effects of Dexamethasone in Unilateral Total Knee Replacement

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The effect of acute pre-surgery dexamethasone treatment on the inflammatory immune and endocrine responses to orthopaedic surgery was investigated. Whole blood samples were obtained before and 5 days after surgery for immune analysis, and serum was obtained before and 6 h, 3 days and 5 days after surgery for endocrine assessment. Dexamethasone did not affect the post-surgery granulocyte response, but inhibited the increase in monocyte count (an average increase of 38.5% was seen in the control group). Peak C-reactive protein concentration (3 days after surgery) was 51.4% lower in the dexamethasone group than in the control group. Dexamethasone had a major effect on cortisol concentrations and the cortisol:testosterone and cortisol: dehydroepiandrosterone ratios, but no effect on anabolic hormone concentrations. In conclusion, acute pre-surgery dexamethasone treatment may have beneficial effects in the post-surgery period, by limiting the extent of systemic inflammation and the cortisol response.

KEY WORDS: Dexamethasone; Cortisol antagonist; Inflammation; Orthopaedic surgery; C-reactive protein; Growth hormone; Dehydroepiandrosterone sulphate (DHEAS); Testosterone

Introduction

Both myofibrillar and whole-body protein catabolism have been shown to increase post-operatively in surgically stressed patients.[1] This increased protein catabolism has been ascribed to the mobilization of skeletal muscle proteins for redistribution to support vital organs, improve resistance to infection and aid wound healing.[2] The hormone ultimately responsible for this post-injury mobilization of protein is the stress hormone cortisol.[3] Although an elevation in circulating cortisol is necessary in the post-operative period, the subsequent catabolic process requires a counteracting process of muscle growth to prevent severe decreases in muscular strength and prolonged convalescence.

However, even at rest, the circulating concentrations of some of these vital anabolic hormones, e.g. dehydroepiandrosterone and growth hormone, have been shown to decline with age, while that of the catabolic hormone cortisol has been reported to be increased in older subjects.[4 – 6] This effect of ageing on the

balance between the anabolic and catabolic arms of the endocrine system may have negative implications for the recovery of elderly patients after surgical procedures.

Dexamethasone is a synthetic cortisol antagonist routinely used to probe central glucocorticoid sensitivity in the diagnosis and treatment of depression.[7] Dexamethasone administration in rats was reported to inhibit the glucocorticoid response by blocking peripheral glucocorticoid receptors (i.e. in the pituitary and spleen), while having no effect on glucocorticoid receptor binding in the brain (hypothalamus, hippocampus and cortex).[8] In another rat study, low-dose dexamethasone administration (0 – 50 µg/kg) was shown to result in a dose-dependent reduction in stress-induced corticosterone concentration in restrained rats, a higher dose of dexamethasone resulting in a greater reduction.[9] Dexamethasone may therefore be a useful tool to prevent an 'overshoot' of the catabolic response to elective surgery, especially in the elderly. Other side-effects experienced post-operatively include pain and nausea, on which the effects of a single dose of dexamethasone treatment have also been investigated. The single dose of dexamethasone administered intravenously during surgery tended to reduce pain in the first few days after surgery in adult patients who had undergone tonsillectomy.[10] A single 8 mg dose of dexamethasone was unsuccessful as an antiemetic.[11] In contrast, in a larger study of 300 patients, 4 mg dexamethasone administered after the induction of anaesthesia, with a second dose on the morning after surgery, significantly reduced nausea and vomiting and improved appetite.[12] This suggests another possible beneficial effect of dexamethasone treatment in the prevention of catabolic processes. In patients undergoing rhinoplasty, a single dose of dexamethasone administered either before or after surgery reduced both upper and lower eyelid oedema for the first 2 days after surgery, suggesting a possible anti-inflammatory effect.[13]

Unfortunately, the effects of altered glucocorticoid action on anabolic hormones or the immune system were not assessed in these studies, so it is unclear what effect dexamethasone administration may have on the balance between pro- and anti-inflammatory responses. A recent review[14] summarized the net effects of stress and ageing on immunity, and proposed a 'double-hit' model for stress and ageing, whereby the age-related increase in the cortisol:dehydroepiandrosterone sulphate (DHEAS) ratio synergizes with stress-induced elevated cortisol to reduce immunity in the elderly significantly. Limiting the cortisol response to surgical stress therefore seems to be a form of management worth investigating. On the other hand, for wound healing to occur, inflammation (i.e. pro-inflammatory cells and cell debris) needs to be cleared from the site of injury. Therefore, despite its negative effects on immunity in the elderly and protein metabolism after surgery, cortisol is required in the post-surgery period for its anti-inflammatory action. It is therefore vital to ensure that the immune cell count and distribution are not altered too much as a result of dexamethasone administration.

The aims of this study were (i) to investigate whether administration of a stat dose of dexamethasone prior to surgery may limit the sharp increase in circulating cortisol reported after surgery; and (ii) to determine the effects of such a treatment on the anabolic endocrine and the immune responses to surgery.

Patients and methods

PATIENTS

Male patients undergoing unilateral total knee replacement surgery were recruited for this study through their orthopaedic surgeon. Subjects undergoing the procedure as a result of malignancy or chronic disease, as well as subjects with a history of endocrine pathology, were excluded from the study. All subjects were informed about the study and gave their written consent prior to participating in the study. Ethical approval for the study was granted by the Stellenbosch University Subcommittee C and the Medi-Clinic ethics boards.

INTERVENTION

All subjects were subjected to similar protocols for epidural anaesthesia and surgical procedures. All knee replacements were carried out by the same orthopaedic surgeon. However, 50% of patients also received a single dose of 16 mg dexamethasone epidurally directly after placement of the epidural catheter. (This dosage was chosen in accordance with that commonly used in elective surgery, to limit undesirable side-effects experienced post-operatively, such as nausea, vomiting, pain and swelling.)

SAMPLE COLLECTION AND ANALYSIS

Whole blood samples were collected by venepuncture into SST Vacutainer® tubes (BD Systems, Plymouth, UK) before surgery and again 6 h, 3 days and 5 days after surgery, for the determination of the serum concentrations of cortisol, testosterone, DHEAS, growth hormone and C-reactive protein (CRP). In addition, blood samples in ethylenediaminetetraacetic acid were collected before surgery and again 5 days after surgery, for the determination of total and differential white blood cell (WBC) counts. With the exception of the 6-h post-surgery samples, all samples were drawn in a fasted, rested state at 08.00, by a registered phlebotomist. Patients consumed only fluids for the first 6 h after surgery. Samples were then transported to the on-site pathology laboratory, where they were processed immediately for all parameters (total and differential WBC, CRP, cortisol, testosterone, growth hormone, DHEAS) by automated analytical procedures (STKS & Access® B module 81600, Beckman/Coulter, Fullerton, California, USA; Immulite® I, Diagnostic Products Corporation, Los Angeles, California, USA; Advia Centaur, Bayer Diagnostics, Leverkusen, Germany).

STATISTICAL ANALYSIS

All results are represented as mean ± SD. The effects of time and/or treatment alone, as well as the interaction effect of both time and treatment, on the selected immune and endocrine parameters were determined by factorial analysis of variance (ANOVA) and Bonferroni *post hoc* tests. *P*-values < 0.05 were considered to be statistically significant.

Results

Twelve male patients, aged 65 ± 9 years, undergoing unilateral total knee replacement surgery were recruited ($n = 6$ in the dexamethasone group and $n = 6$ in the control group). ANOVA indicated an effect of time ($P < 0.005$) on total and differential WBC counts, but no effect of dexamethasone treatment on these parameters. Bonferroni *post hoc* testing indicated similar significant increases in both the control and dexamethasone groups for both total WBC and neutrophil counts 5 days after surgery ($P < 0.05$); the monocyte count was significantly increased from pre-surgery values at the same time-point, but only in the control group ($P < 0.05$) (Table 1). A main effect of

TABLE 1:
Changes in total and differential white blood cell (WBC) counts over time after unilateral knee replacement surgery in 12 male subjects with or without a pre-surgery single dose of 16 mg dexamethasone administered epidurally ($n = 6$ per group)

Parameter	Normal range	Group	Before surgery	5 days after surgery
WBC ($\times 10^9$/l)	4.00 – 11.00	Control	7.18 ± 0.81	8.90 ± 2.38[a]
		Dexamethasone	7.22 ± 1.87	9.22 ± 3.20[a]
Neutrophils ($\times 10^9$/l)	2.50 – 7.50	Control	4.23 ± 0.74	6.08 ± 1.84[a]
		Dexamethasone	4.27 ± 1.11	6.22 ± 2.11[a]
Lymphocytes ($\times 10^9$/l)	1.50 – 3.50	Control	2.02 ± 0.53	1.60 ± 0.54
		Dexamethasone	1.99 ± 0.62	1.93 ± 0.85
Monocytes ($\times 10^9$/l)	0.20 – 0.80	Control	0.65 ± 0.23	0.90 ± 0.33[a]
		Dexamethasone	0.70 ± 0.23	0.71 ± 0.33

Values are mean ± SD.
Groups were compared using main effects analysis of variance with Bonferroni *post hoc* tests.
[a]$P < 0.05$ compared with the before-surgery value in the same group.
No inter-group differences were evident before or after surgery.

time was reported for CRP concentration ($P < 0.00001$), as well as a main effect of treatment, indicating that the elevations in CRP over time differed in the dexamethasone group ($P < 0.001$). Specifically, the interaction effect between time and treatment ($P < 0.005$) and the Bonferroni *post hoc* analysis illustrated a lower peak CRP concentration in the dexamethasone group 3 days after surgery (Fig. 1).

Similarly, factorial ANOVA indicated main effects of time ($P < 0.05$) and treatment ($P < 0.0005$) on serum cortisol concentration. However, the Bonferroni *post hoc* analysis indicated that the significantly lower cortisol concentration in the dexamethasone group was evident at an earlier time-point than CRP, namely 6 h after surgery ($P < 0.01$) (Fig. 2A). The anabolic hormones analysed did not follow the same responses as those seen for cortisol and CRP. Although testosterone concentration was affected by time ($P < 0.0005$), it was not affected by treatment (Fig. 2B). Serum concentrations of DHEAS and growth hormone did not seem to be affected by either treatment or time (Table 2). Taking into account that the net catabolic status may be influenced by the ratio between catabolic and anabolic variables, ratios between cortisol and testosterone and between cortisol and DHEAS were also calculated. First, it was noticeable that the large variability in response between subjects (large standard deviations) for both cortisol and testosterone was greatly diminished by assessing the ratio between these two parameters in the early response to the surgical intervention (Figs 2A – 2C). The variability between subjects was evident again 5 days after surgery. Also, a dramatic effect of time was no longer evident. Rather, ANOVA indicated main effects of treatment on cortisol:testosterone ($P < 0.05$; Fig. 2C) and cortisol:DHEAS ($P < 0.01$; Table 2) ratios, with values lower in the dexamethasone group compared with the control group for both variables.

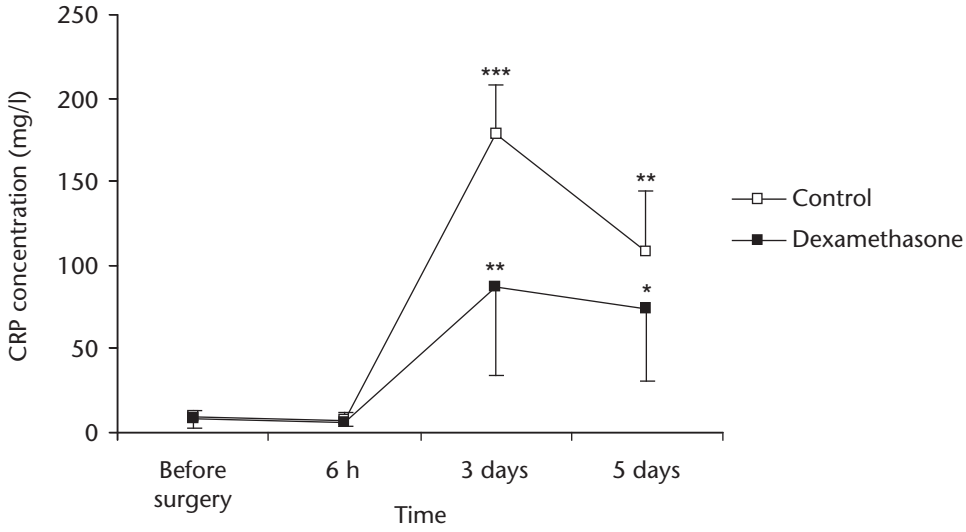

FIGURE 1: Serum C-reactive protein (CRP) concentrations before and after unilateral knee replacement in 12 male subjects, with or without a pre-surgery single dose of 16 mg dexamethasone administered epidurally ($n = 6$ per group). ***Value different from all other values ($P < 0.005$); **value different from all values before surgery and 6 h after surgery, and from control at the indicated number of days after surgery ($P < 0.005$); *value different from all values before surgery and 6 h after surgery, and from control at the indicated number of days after surgery ($P < 0.05$). Values are mean ± SD

TABLE 2:
Changes in hormone concentrations and ratios after unilateral knee replacement surgery in 12 male subjects with or without a pre-surgery single dose of 16 mg dexamethasone administered epidurally ($n = 6$ per group)

Parameter	Group	Before surgery	6 h after surgery	3 days after surgery	5 days after surgery
DHEAS (µmol/l)	Control	2.07 ± 0.83	1.65 ± 0.61	1.55 ± 0.49	2.20 ± 2.03
	Dexamethasone	2.50 ± 1.17	1.63 ± 0.87	1.20 ± 0.56	1.35 ± 0.66
Growth hormone (µIU/ml)	Control	3.18 ± 3.79	2.34 ± 1.48	2.15 ± 2.70	3.38 ± 3.61
	Dexamethasone	2.24 ± 4.83	3.46 ± 1.77	1.79 ± 2.03	1.55 ± 1.25
Cortisol:DHEAS ratio	Control	0.20 ± 0.12	0.49 ± 0.35	0.21 ± 0.07	0.41 ± 0.29
	Dexamethasone	0.11 ± 0.07	0.08 ± 0.10[a]	0.12 ± 0.08	0.37 ± 0.21

Values are means ± SD.
Analysis of variance shows the effect of time on cortisol:dehydroepiandrosterone sulphate (DHEAS) ratio ($P < 0.01$).
Bonferroni *post hoc* analysis: [a]$P < 0.05$ compared with the control group.

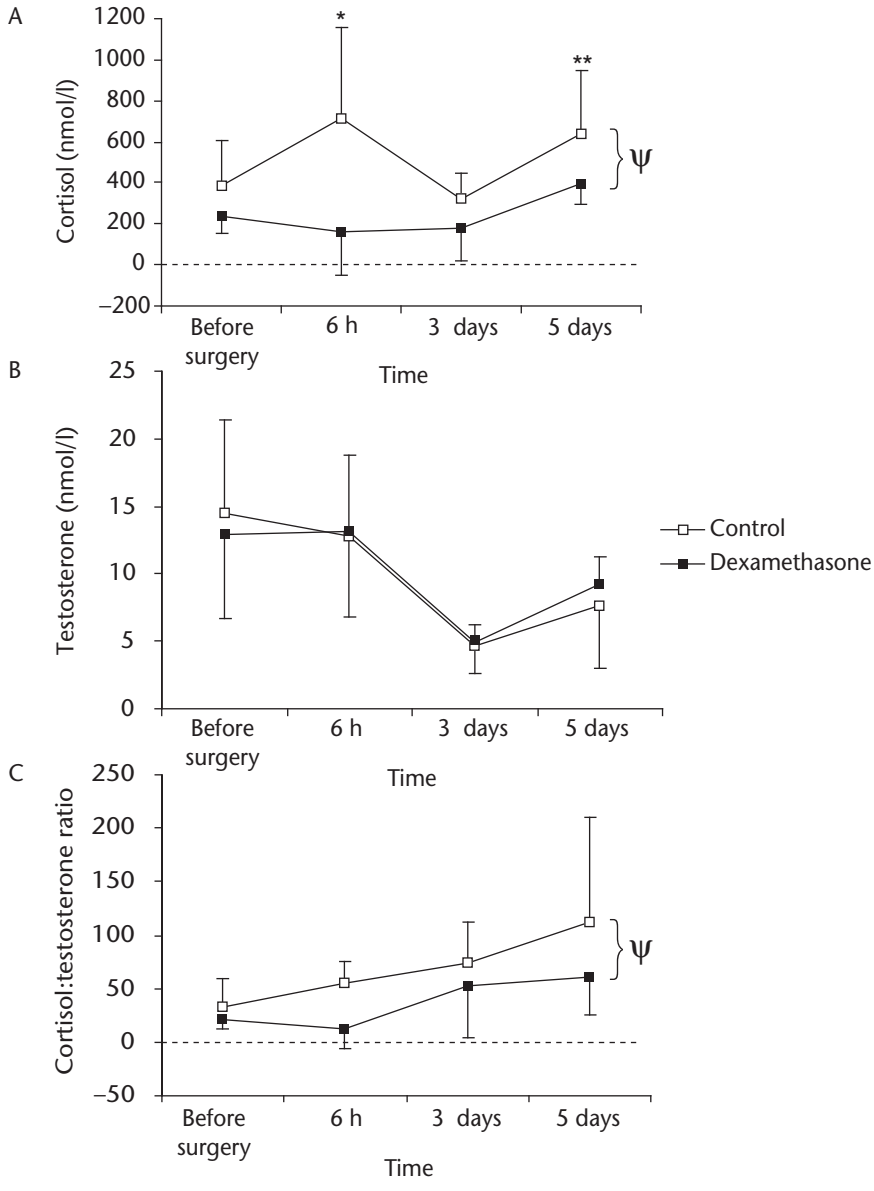

FIGURE 2: Effect of unilateral knee replacement surgery in 12 patients, with or without a pre-surgery single dose of 16 mg dexamethasone administered epidurally (n = 6 per group), on the circulating concentrations of cortisol and testosterone. (A) Cortisol. Ψ, ANOVA, effect of treatment, values for the dexamethasone group are significantly lower compared with the control group ($P < 0.0005$); *value significantly different from values before surgery and at 6 h and 3 days after surgery in the dexamethasone group ($P < 0.01$); **value significantly different from values at 6 h and 3 days after surgery in the dexamethasone group ($P < 0.05$). (B) Testosterone. (C) Cortisol:testosterone ratio. Ψ, ANOVA, effect of treatment, values are lower in the dexamethasone group than in the control group ($P < 0.05$). Values are mean ± SD

Discussion

The main novel finding of this study was that administration of a single moderate dose of dexamethasone prior to orthopaedic surgery prevented a post-surgery increase in monocyte count. The data also illustrate the following additional main effects: (i) limitation of peak CRP concentrations 3 days after surgery; and (ii) attenuation of the acute post-surgery increase in circulating cortisol without affecting serum levels of the anabolic hormones assessed (testosterone, DHEAS and growth hormone).

The stress of orthopaedic surgery results in a systemic inflammatory response. This inflammatory response is the result of cumulative effects of, for example, anaesthesia,[15] autologous infusion of filtered whole blood[16] and mechanical stress and tissue damage caused by the surgical procedure itself. The finding of increased total WBC and neutrophil counts in the post-surgery period is therefore not unexpected. In addition, these results indicate that administration of a single moderate dose of dexamethasone prior to surgery does not affect this post-surgery granulocyte response. However, dexamethasone did inhibit the monocyte response that was seen in the control group and this lasted up to 5 days after surgery. This novel *in vivo* finding is similar to the findings of an earlier study, which reported monocytopenia within 4 – 6 h after oral administration of a single dose of 12 mg dexamethasone.[17] Taken together, these data suggest that the anti-inflammatory effect of dexamethasone may be limited to the mononuclear cell lines, the granulocyte cell lines being unaffected.

The role of neutrophils after surgery is to phagocytose any debris and damaged cell matter that result from the surgical procedure, and it is therefore important to note that this positive role is not inhibited by dexamethasone treatment. Monocyte infiltration is, in turn, necessary to clear these neutrophils from the injury site, so that healing may occur. There is more than one possible explanation for the unchanged monocyte count seen in the circulation in the dexamethasone group. First, it may indicate better infiltration of monocytes from the circulation into damaged tissue. However, since a study in rats illustrated no effect of dexamethasone treatment on monocyte (macrophage) infiltration into a tumour, and even inhibition of microglia and lymphocyte infiltration,[18] this explanation is unlikely. A second explanation is that the unchanged circulating monocyte count may be the result of a lack of proliferation or a lack of mobilization of monocytes. Whether or not dexamethasone affects peripheral blood mononuclear cells (of which monocytes form a part) has been addressed in several other studies which have recently been reviewed.[19] The review assessed both clinical studies in which patients were treated with dexamethasone and *in vitro* studies in which blood samples were incubated with dexamethasone. The authors concluded that dexamethasone treatment has an inhibitory effect on the activation of peripheral blood mononuclear cells. Therefore, the inhibited monocyte response is likely to be due to a dexamethasone-induced reduction in cell proliferation.

Whether the lack of elevation in monocyte count in the dexamethasone group might lead to slower clearance of inflammation remains to be investigated. The fact that the CRP levels did not change between 3 and 5 days after surgery in the dexamethasone group could be interpreted as indirect evidence of this delay. In contrast, administration of dexamethasone decreased the peak concentration of CRP seen in circulation 3 days after surgery. This

result implies that the peak inflammatory response was attenuated. Although assessed in circulation, this may be indicative of a lower peak magnitude of inflammation at the tissue level in response to the surgery in the dexamethasone group than in the control group.

A potentially beneficial effect of dexamethasone, which is illustrated in this study, is the limitation of the catabolic process induced by cortisol. Although it is known from rat studies that dexamethasone suppresses cortisol secretion by inhibition of the hypothalamo–pituitary–adrenal axis at the level of the pituitary glucocorticoid receptors,[8,9] this study is the first in human subjects to show a suppressed cortisol response to knee replacement surgery after a single dose of dexamethasone. Concentrations of the anabolic hormones testosterone, DHEAS and growth hormone were not affected by dexamethasone treatment. This resulted in a net reduction in the ratios of catabolic to anabolic hormones. This suggests that dexamethasone blocks the action of cortisol without affecting the anabolic pathway. Therefore, this action of dexamethasone may suggest a treatment for the prevention of surgery-induced catabolic states without the side-effects generally associated with exogenous administration of anabolic hormones.[20,21]

In conclusion, these results indicate a beneficial effect of a single moderate dose of dexamethasone, administered prior to orthopaedic surgery, for the maintenance of anabolic versus catabolic endocrine action. Furthermore, the use of dexamethasone seems to decrease the magnitude of inflammation at the site of surgery. The potential benefits of these effects may be outweighed in part by slower clearance of inflammation later, but this remains to be investigated in more detail. Also, the possible downstream effects on the maintenance of skeletal muscle mass and recovery time after surgery warrant further investigation.

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Conflicts of interest

No conflicts of interest were declared in relation to this article.

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