Original Article

The effect of pre-operative methylprednisolone on early endothelial damage after total knee arthroplasty: a randomised, double-blind, placebo-controlled trial

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Summary

We wished to evaluate whether inhibition of the systemic inflammatory response by a single pre-operative dose of methylprednisolone reduced markers of early endothelial damage after fast-track total knee arthroplasty. We randomly allocated 70 patients undergoing elective unilateral total knee arthroplasty (1:1) to receive either pre-operative intravenous methylprednisolone 125 mg (methylprednisolone group) or isotonic saline (control group). All procedures were performed under spinal anaesthesia without a tourniquet, using a standardised multimodal analgesic regime. The outcomes included changes in Syndecan-1 concentrations, a marker of glycocalyx degradation, markers of endothelial cell damage and activation (plasma soluble thrombomodulin and sE-Selectin), and permeability by vascular endothelial growth factor, as well as C-reactive protein concentrations. Blood samples were collected at baseline and 2 h, 6 h and 24 h after surgery, with complete sampling from 63 patients for analyses. Methylprednisolone significantly reduced markers of endothelial damage at 24 h following surgery compared with saline (methylprednisolone group vs. control group, adjusted means (SEM)) expressed by circulating Syndecan-1: 11.6 (1.0) $ng.ml^{-1}$ vs. 13.4 (1.1) $ng.ml^{-1}$ $p = 0.046$; soluble thrombomodulin: 5.1 (0.1) $ng.ml^{-1}$ vs. 5.7 (0.2) $ng.ml^{-1}$, $p = 0.009$; sE-Selectin: 64.8 (1.8) $ng.ml^{-1}$ vs. 75.7 (1.9) $ng.ml^{-1}$, $p = 0.001$, and vascular endothelial growth factor: 35.3 (2.7) $ng.ml^{-1}$ vs. 58.5 (2.8) $ng.ml^{-1}$, $p < 0.001$. The effect of the intervention increased with time for soluble thrombomodulin, sE-Selectin and vascular endothelial growth factor, and was more pronounced in patients with high baseline values. Finally, methylprednisolone reduced the C-reactive protein response 24 h postoperatively; 31.1 (1.1) $mg.l^{-1}$ vs. 68.4 (1.1) $mg.l^{-1}$, $p < 0.001$. Pre-operative administration of methylprednisolone 125 mg reduced circulating markers of endothelial activation and damage, as well as the systemic inflammatory response (C-reactive protein) early after fast-track total knee arthroplasty. These findings may have a positive effect on surgical outcome, but require studies in major surgery.

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Introduction

Total knee arthroplasty (TKA) is followed by a surgical stress response, where the inflammatory response is significant [1]. Surgical trauma and accompanying inflammation result in increased capillary permeability leading to tissue oedema [2]. Since the vascular endothelium contributes to homeostasis [3], endothelial damage may increase the risk of cardiovascular and thromboembolic complications [4]. The endothelium comprises of a single layer of cells that lines all blood vessel, covering a total surface area of 4000–7000 m^2, and weighing a total of 1 kg [5]. The glycocalyx is a 0.2 to 1-μm-thick negatively charged anti-adhesive and anticoagulant carbohydrate-rich surface layer that covers and protects the endothelial cells, and helps to maintain the integrity of the vascular barrier [6]. Disturbances of the endothelial glycocalyx can range from discrete damage to the composition of the inner-most luminal layers, to excessive destruction resulting in loss of the entire glycocalyx [6]. The endothelial glycocalyx may play an important role in the post-injury inflammatory response [7], and increases in C-reactive protein (CRP) levels are associated with decreased thickness of the endothelial glycocalyx, and impaired endothelial vasoreactivity [8, 9]. Syndecan-1 appears to be a sensitive marker of early damage to the endothelial glycocalyx [10]. Endothelial disruption following injury may have detrimental consequences on outcome after trauma and critical illness [4, 11–15].

Glucocorticoids are known to reduce the systemic inflammatory response after surgery [1, 16], and have well-documented anti-inflammatory effects [17]. Animal studies have shown that glucocorticoids may reduce tissue oedema formation and shedding of glycocalyx caused by Tumour necrosis factor-α [18, 19], but no clinical data are available. Consequently, potential restoration or maintenance of the glycocalyx by glucocorticoids following acute trauma, critical illness and surgery is of interest.

We wished to evaluate whether a single pre-operative dose of methylprednisolone reduced the shedding of endothelial glycocalyx markers 24 h after fast-track TKA, as assessed by plasma Syndecan-1 (CD138), as well as plasma soluble thrombomodulin (sTM) (CD141), plasma sE-Selectin (CD62E) and vascular endothelial growth factor (VEGF), which are biomarkers of endothelial cell injury, activation and permeability, respectively.

Methods

We conducted this single-centre, randomised, placebo-controlled superiority trial with two parallel groups, with blinding of the participants, intervention deliverers and outcome assessors. The trial was approved by the Ethics Committee for the Capital Region of Denmark, the Danish Data Protection Agency, the Danish Health and Medicine Authority, and was registered at ClinicalTrials.gov under the US. National Library of Medicine, embedded in a primary study on the effect of glucocorticoids on postoperative quadriceps dysfunction. The trial was conducted according to the principles of the Helsinki Declaration, and was monitored by the GCP (Good Clinical Practice) unit of the Copenhagen University Hospital, Copenhagen, Denmark. Oral and written informed consent was obtained from all patients before participation, and we followed the CONSORT recommendations for reporting randomised, controlled clinical trials [20].

We assessed consecutive patients undergoing elective, unilateral, primary TKA at Copenhagen University Hospital Bispebjerg and Frederiksberg, Denmark, for eligibility during the period February 2015 to April 2016. Inclusion criteria were the ability to speak and understand Danish, and to provide informed oral and written consent. The exclusion criteria were: age < 55 years and > 80 years; general anaesthesia; allergy towards glucocorticoids; daily use of systemic glucocorticoids; local or systemic infection; insulin-dependent diabetes mellitus; treatment of peptic ulcer within 30 days from inclusion; cancer; autoimmune disease including rheumatoid arthritis; and fertile women.

We randomly allocated 70 of the included patients – after the baseline assessment of outcomes – to two groups of 35. A computer-generated random allocation sequence (1:1 allocation rate) concealed in 70 consecutively numbered, opaque, sealed envelopes determining active treatment or placebo was generated by a research assistant not otherwise involved in the trial. The envelopes were opened consecutively on the morning of surgery, and the trial drug was prepared by two anaesthetic nurses not otherwise involved in

the collection of patient data. The patients received either a single dose of intravenous (i.v.) methylprednisolone, 125 mg (2 ml) (Solu-Medrol®; Pfizer, Ballerup, Denmark) (methylprednisolone group), or a single dose of i.v. isotonic saline (2 ml) (control group). The syringes were masked, even though both solutions were transparent and identical in appearance. The test solution was administered by the principal investigator just after spinal anaesthesia. Trial participants, care providers, data collectors and investigators were all blinded to the allocation. After study termination, the blinded randomisation list was dispatched to the primary investigator, enabling blinded analyses. This list was unblinded with respect to intervention type only after all statistical analyses had been carried out.

We followed standard procedures for anaesthesia, surgery and analgesia. About 1 h before surgery, patients received oral paracetamol 1 g and naproxen 500 mg. All surgeries were performed under lumbar spinal anaesthesia with 7.5–12.5 mg of hyperbaric bupivacaine (5 $mg.ml^{-1}$, 0.5%). We administered additional sedation with propofol (1–5 $mg.kg^{-1}.h^{-1}$), as required. We used cefuroxime 1.5 g for infectious prophylaxis and tranexamic acid 1 g for control of haemostasis intravenously, immediately after spinal anaesthesia. Intra-operative fluid therapy was standardised, and consisted of 0.9% saline 12 $ml.kg^{-1}.h^{-1}$ in the first hour of surgery, followed by 6 $ml.kg^{-1}.h^{-1}$ if the surgery was prolonged beyond 1 h. Spinal anaesthesia-induced hypotension was treated at the discretion of the attending anaesthetist with either ephedrine 10 mg i.v., or with phenylephrine 0.1–0.2 mg i.v.

Total knee arthroplasty was performed using a standard medial parapatellar approach, without the use of a femoral tourniquet. Local infiltration analgesia was performed intra-operatively, with 150 ml ropivacaine 0.2% injected at the end of surgery. In the recovery area and in the ward, patients were allowed to drink freely. Thromboprophylaxis with rivaroxaban (Xarelto, Bayer Pharma, Berlin, Germany) 10 $mg.day^{-1}$ was given 6–8 h after surgery. Patients followed a routine, well-defined, fast-track rehabilitation regime [21], and received oral paracetamol 1 g four times daily and naproxen 500 mg twice daily from the day of surgery. Opioids were administered as rescue analgesia on request, if pain exceeded numeric rating scale (0–10) of 3 at rest, or 5 during active movement. We treated postoperative nausea and vomiting with ondansetron 4 mg, and all patients received zolpidem 10 mg at night.

The primary outcome was change in plasma Syndecan-1 concentration from baseline to 24 h postoperatively. Secondary outcomes included changes in plasma soluble thrombomodulin (sTM), plasma sE-Selectin and plasma VEGF, as well as plasma CRP concentrations. Outcomes were assessed pre-operatively (baseline), and 2 h, 6 h and 24 h postoperatively, respectively.

We collected blood in ethylenediamine tetra-acetic (EDTA) tubes upon arrival at the operating theatre (baseline, T0), and at 2 h (T2), 6 h (T6) and 24 h (T24) postoperatively. The samples were placed on ice immediately, and centrifuged at 3000 g for 10 min within 30 min of collection. Plasma was stored in a −80° C freezer awaiting analyses. Soluble biomarkers of endothelial glycocalyx (Syndecan-1) [10], endothelial cell damage (sTM) [22], endothelial cell activation (sE-Selectin) [23] and endothelial permeability (VEGF) [24] were measured by commercially available immunoassays in EDTA plasma. Syndecan-1 (Diaclone Nordic Biosite, Copenhagen, Denmark) had a lower limit of detection (LLD) of 0.31 $ng.ml^{-1}$, soluble thrombomodulin (sTM, Nordic Biosite) a LLD of 4.94 $ng.ml^{-1}$, sE-Selectin (R&D Systems Europe, Abingdon, UK) a LLD of 0.009 $ng.ml^{-1}$ and VEGF (R&D Systems Europe) a LLD of 3.5 $pg.ml^{-1}$. The enzyme-linked immunosorbent assay analyses were conducted by trained laboratory technicians at the Haemostasis Research Laboratory, Section for Transfusion Medicine, Rigshospitalet, Copenhagen, Denmark.

We based the sample size calculation on the primary study on the effect of glucocorticoids on quadriceps function (NCT02319343), which required a total sample size of 52 patients, and allowing for a 25% drop-out rate, we included a total of 70 patients in the trial. The present biomarker study was considered explorative and hypothesis-generating, since no previous clinical data were available.

Before the analyses, we validated all data by double entry, and evaluated for normal distribution by histograms and Q-Q plots. Syndecan-1 and CRP data did

not follow normal distribution and underwent log transformation before statistical analysis. There were no missing data regarding patient characteristics or individual biomarker measurements. We compared baseline marker values using an independent sample t-test, and evaluated postoperative changes from baseline in marker concentrations within groups using a paired sample t-test. A linear mixed model was used for exploring overall time trends in the repeated marker measurements. We compared biomarker concentrations between allocation groups using a univariate linear regression analysis incorporating baseline values as covariate (adjusted means), investigating each sampling time point [25]. The adjusted means represent estimated marginal means, that is, model-predicted means when having the same baseline (T0) value, thereby adjusting for individual differences in baseline values [25] and simplifying evaluation of the intervention effect.

We performed statistical analyses using SPSS version 22.0 (IBM Corp, Troy, NY, USA), and considered a two-sided p value < 0.05 to be statistically significant.

Results

The CONSORT diagram is shown in Fig. 1. We assessed 163 patients for eligibility from February 2015 to April 2016. Of these, 93 were not eligible or did not consent to participate in the study, leaving 70 patients for randomisation resulting in an inclusion rate of

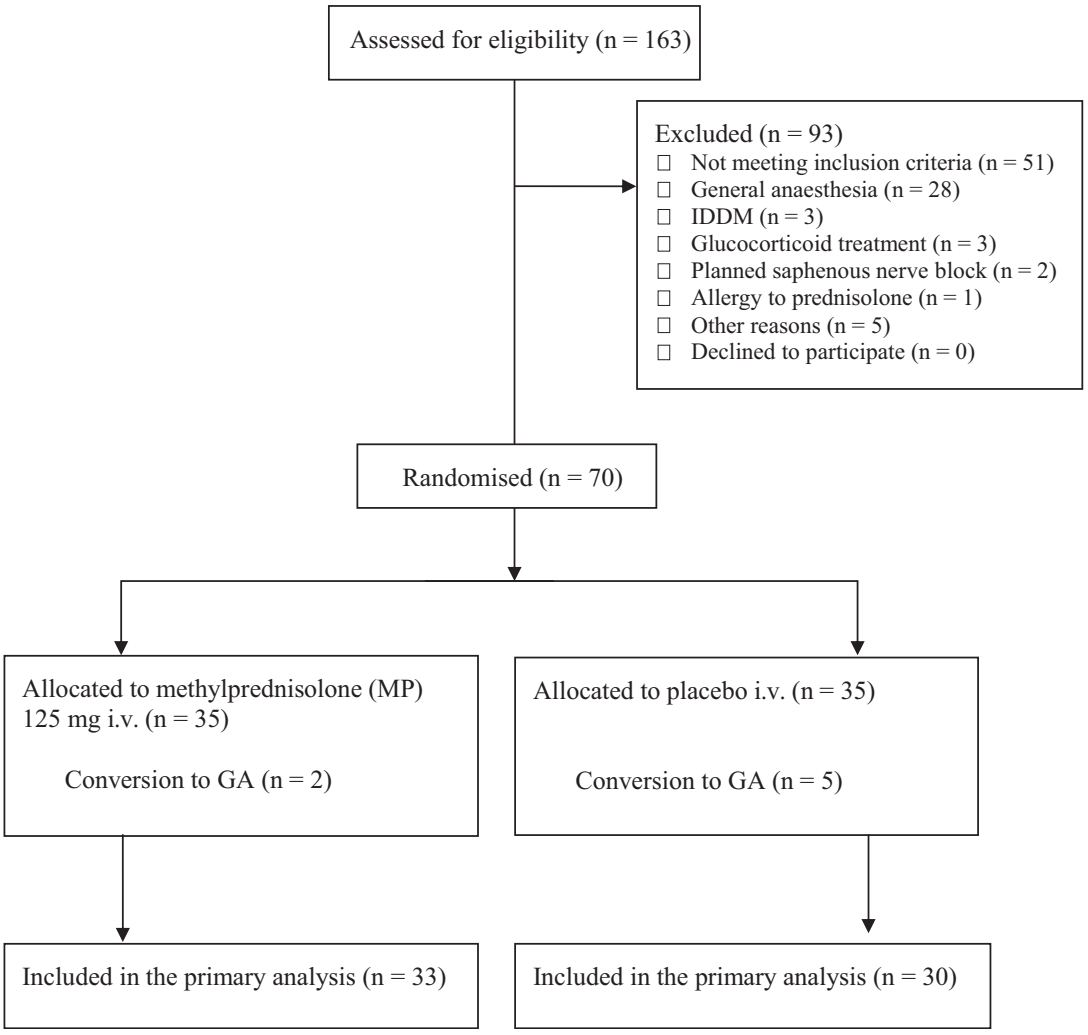

Figure 1 CONSORT diagram. GA, general anaesthesia; IDDM, insulin-dependent diabetes mellitus, i.v., intravenous.

43%. Seven patients were excluded following randomisation, five before receiving the trial drug due to planned conversion to general anaesthesia, and two after receiving the trial drug, but converted to general anaesthesia due to insufficient spinal anaesthesia. Consequently, 33 (methylprednisolone group) and 30 (control group) patients were available for analysis of the primary outcome measures using the non-missing scores only. Baseline characteristics were comparable between allocation groups (Table 1). Likewise, there were no between-group differences in intra-operative data (Table 1). No patient received blood components during or after surgery.

Baseline and within-group postoperative changes in endothelial biomarkers are shown in Table 2. Baseline values were similar between groups, except for Syndecan-1, where four patients in the control group had values > 75 ng.ml^{-1}, skewing the baseline results ($p = 0.007$) for that variable (Table 2), but without differences between the other endothelial markers sTM ($p = 0.981$), sE-Selectin ($p = 0.279$), VEGF ($p = 0.181$) or CRP ($p = 0.373$). When leaving out the four Syndecan-1 outliers, we found no differences between baseline values ($p = 0.954$). We decided to include the outliers in the following analyses as we considered them to be an expression of natural variation. Over time, Syndecan-1 concentrations remained stable in the control group, with a fall in the methylprednisolone group. Soluble thrombomodulin increased in the control group, but only transiently in the methylprednisolone group. sE-Selectin decreased in both groups, but more so in the methylprednisolone group. Vascular endothelial growth factor increased in the control group, but only transiently in the methylprednisolone group. C-reactive protein increased in both groups, but less so in the methylprednisolone group ($p < 0.001$). The effect of methylprednisolone on sTM, sE-Selectin and VEGF concentrations was more

Table 1 Baseline and procedural characteristics of 63 patients undergoing total knee arthroplasty and randomly allocated to pre-operative intravenous (i.v.) methylprednisolone or control (isotonic saline) groups. Values are mean (SEM) or number (proportion).

	Methylprednisolone group n = 33	Control group n = 30
Age; years	65.0 (1.2)	67.7 (1.3)
Sex; female	20 (61%)	15 (50%)
BMI; kg.m^{-2}	31.5 (1.0)	30.1 (1.0)
ASA physical status		
1	10 (30%)	4 (14%)
2	23 (70%)	23 (76%)
3	-	3 (10%)
Duration of surgery; min	57 (3)	60 (3)
Spinal bupivacaine dose; mg	11.7 (0.2)	11.3 (0.2)
Intra-operative propofol sedation; mg	208 (39)	217 (29)
Crystalloids; ml	1016 (43)	1022 (55)
HES	0	0
Packed erythrocytes	0	0
Blood loss; ml	117 (23)	128 (18)

BMI, body mass index; ASA, American Society of Anesthesiologists; HES, hydroxyethyl starch.

Table 2 Unadjusted means for endothelial marker concentrations at different time points in patients undergoing total knee arthroplasty and randomly allocated to pre-operative intravenous (i.v.) methylprednisolone or control (isotonic saline) groups. Values are presented as mean (SEM).

	Methylprednisolone group n = 33	p value	Control group n = 30	p value
Syndecan-1*; ng.ml^{-1}				
T0	14.1 (1.4)		25.4 (3.2)	
T2	13.5 (2.2)	0.185	23.5 (5.3)	0.006
T6	13.5 (2.0)	0.993	25.0 (5.4)	0.243
T24	12.4 (2.0)	0.001	25.3 (5.4)	0.813
Soluble thrombomodulin; ng.ml^{-1}				
T0	5.0 (0.2)		5.0 (0.2)	
T2	5.3 (0.4)	0.035	5.0 (0.2)	0.828
T6	5.2 (0.4)	0.022	5.4 (0.3)	0.008
T24	5.1 (0.3)	0.499	5.6 (0.3)	< 0.001
sE-Selectin; ng.ml^{-1}				
T0	87.3 (3.3)		93.1 (4.2)	
T2	80.5 (5.3)	< 0.001	79.2 (6.3)	< 0.001
T6	74.5 (5.6)	< 0.001	79.1 (6.4)	< 0.001
T24	62.8 (4.2)	< 0.001	78.0 (6.1)	< 0.001
VEGF; ng.ml^{-1}				
T0	37.7 (2.5)		42.4 (2.5)	
T2	45.9 (6.0)	0.051	40.9 (3.5)	0.656
T6	47.0 (4.9)	0.019	54.8 (4.1)	0.008
T24	34.2 (3.1)	0.247	58.9 (3.3)	0.002
CRP*; mg.l^{-1}				
T0	4.5 (1.1)		6.9 (2.2)	
T2	4.4 (1.1)	0.992	6.4 (2.0)	0.037
T6	4.8 (1.1)	0.002	7.3 (2.2)	0.002
T24	36.6 (3.6)	< 0.001	74.4 (5.0)	< 0.001

*Syndecan-1 and CRP analyses are carried out using log-transformed data due to the lack of normal distribution.
T0, baseline; T2/T6/T24, hours postoperatively; VEGF, vascular endothelial growth factor; CRP, C-reactive protein.

pronounced the higher the baseline values ($p = 0.012$, $p = 0.009$ and $p < 0.001$, respectively), but this was not the case regarding Syndecan-1.

In the between-group analysis adjusted for baseline values, all markers of endothelial dysfunction were lower at T24 in the methylprednisolone group compared with the control group (Fig. 2).

There were no wound complications, deep infections or clinically apparent episodes of venous thromboembolism or other adverse events in either group during the study period.

Discussion

To our knowledge, the effects of high-dose glucocorticoid administration on markers of endothelial glycocalyx degradation after elective surgery have not previously been studied in humans. We hypothesised that the administration of a single pre-operative dose of systemic methylprednisolone 125 mg would reduce the inflammatory response to surgery [26], and thereby stabilise the glycocalyx 24 h after TKA surgery. The principal findings of this study were that markers of endothelial integrity deteriorated following surgery, but less so after a single high-dose bolus of i.v. methylprednisolone. Our results support the findings of the animal study by Chappell et al. [18] that glucocorticoids prevented inflammatory shedding of the endothelial glycocalyx, and thereby stabilised endothelial barrier function.

The effects of methylprednisolone on Syndecan-1, sTM and VEGF concentrations depended on the time of sampling ($p = 0.015$, $p = 0.001$, and $p < 0.001$, respectively), as the effects of methylprednisolone increased over time, and were more pronounced the higher the baseline values of the biomarkers (with the exception of Syndecan-1). This may be important, as it allows for the development of personalised interventions targeting patients in selected groups with altered risk profiles, for example, using bolus i.v. methylprednisolone in patients with high baseline values of endothelial activation and damage. The endothelial damage demonstrated by the circulating biomarkers measured has previously been shown to have negative prognostic value for outcome in trauma and during critical illness [4, 11–15]. A high level of Syndecan-1 in trauma patients on admission was found to be associated with high sympathoadrenal activity and increased mortality [3]. Patients with more pronounced endothelial degradation (high Syndecan-1 levels) also demonstrated more profound endothelial damage as assessed by high sTM levels at all sampling

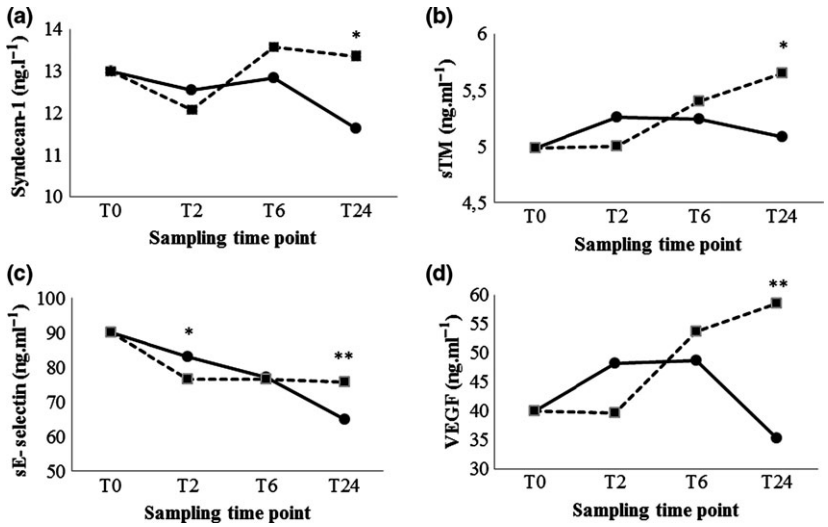

Figure 2 Adjusted means for endothelial marker concentrations at different time points in patients undergoing total knee arthroplasty, and randomly allocated to pre-operative intravenous (i.v.) methylprednisolone or control (isotonic saline) groups: Syndecan-1 (a); soluble thrombomodulin (sTM) (b); sE-Selectin (c); and vascular endothelial growth factor (VEGF) (d). Significant differences between groups by univariate analysis incorporating baseline values as covariate [25] are marked with * (< 0.05) or ** (< 0.001). Control group (■), methylprednisolone group (●).

time points [3]. It is notable that a positive correlation between Syndecan-1 and sTM also existed 24 h postoperatively in the control group in our study, but this correlation was not present in the methylprednisolone group (data not shown).

Glycocalyx degradation has been closely linked to hyperinflammation in trauma patients [3]. An experimental study by Devaraj et al. [8] found that CRP impaired the integrity of the glycocalyx, and was associated with increased endothelial dysfunction. In our study, we found no correlation between CRP (a surrogate marker of inflammation) and any of the endothelial biomarkers in either group we investigated – possibly reflecting the relatively minor contribution of trauma to our study. Increases in endothelial and inflammatory biomarkers might be more pronounced after surgical procedures with more extensive surgical trauma, and require further study comparing endothelial function and relevant clinical outcomes.

The strengths of our study include the high degree of standardised anaesthetic, surgical and multimodal analgesic regime. All patients received spinal anaesthesia using bupivacaine only, thus avoiding intrathecal opioids. The same surgical approach was used, and local anaesthetic was infiltrated in all patients. In addition, the use of only one data collector may have minimised the risks of bias. Limitations include a short follow-up period and lack of power to determine clinical outcomes, neither of which was the purpose of this exploratory study. Another limitation is that we measured circulating biomarkers of glycocalyx and endothelial function from human plasma samples. The major source of these proteins is the endothelium, but some of the glycocalyx components are also present on epithelial cells, complicating the determination of their exact origin. Finally, the selection of biomarkers analysed in this study does not exclude the importance of other markers in the development and progression of endothelial dysfunction. Large-scale dose-finding studies in major surgery are necessary to explore and define the clinical value of glucocorticoid-induced protection of the endothelial glycocalyx.

In conclusion, this placebo-controlled study showed that high-dose pre-operative glucocorticoid administration is associated with reduced endothelial damage in patients undergoing TKA, together with a reduction in the systemic inflammatory response. These findings should be investigated in major procedures, where endothelial damage may have severe consequences.

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