Prostanoid production and pulmonary hypertension after fat embolism are not modified by methylprednisolone

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Bilateral cemented arthroplasty (BCA) in anaesthetized mongrel dogs produces particulate fat and marrow embolism of the lung. Methylprednisolone sodium succinate (MPSS) has been advocated for post-traumatic fat embolism to prevent acute lung injury. We used the BCA procedure to produce acute fat and marrow embolism, and tested the efficacy of MPSS (30 mg · kg⁻¹) in preventing physiological and pathological markers of acute lung injury. Dogs (n = 6) pre-treated with MPSS demonstrated similar acute increases in pulmonary artery pressure (PAP) within one minute of BCA (17.8 ± 7.3 mmHg) as the untreated (control n = 7) dogs (18.6 ± 12.6). Pulmonary vascular resistance (PVR) increased to the same degree in both groups (455 ± 323 and 319 ± 137 dyne · sec · cm⁻⁵) and PaO₂ decreased by 18.3 ± 6.4 mmHg in the control group as opposed to 12.4 ± 7.7 mmHg in the MPSS group within five minutes of BCA. Circulating arterial and mixed venous plasma concentrations of thromboxane B₂ (TxB₂) increased within one minute of BCA in both groups with no increase in the transpulmonary gradient. Arterial plasma 6-keto prostaglandin F_{1α} (6-keto PGF_{1α}) increased (0.91 ± 0.29 ng · ml⁻¹ and 0.87 ± 0.43 ng · ml⁻¹) in both groups one minute after BCA. Mixed venous 6-keto PGF_{1α} plasma concentration also increased, but a significant transpulmonary 6-keto PGF_{1α} gradient was found. We conclude that acute fat and marrow embolism after BCA is

associated with increased levels of TxB₂ and 6-keto PGF_{1α}. The source of the 6-keto PGF_{1α} after acute pulmonary fat embolism is the lung and neither the acute haemodynamic changes nor the prostanoid response are inhibited by MPSS.

L'arthroplastie bilatérale cimentée (BCA) chez des chiens métis anesthésiés produit des embolies pulmonaires graisseuses et de moëlle. Le succinate de méthylprednisolone sodique (MPSS) a été suggéré pour prévenir l'atteinte pulmonaire aiguë lors des embolies pulmonaires graisseuses posttraumatiques. Nous avons utilisé la procédure BCA pour produire des embolies aiguës graisseuses et de moëlle et nous avons examiné l'efficacité du MPSS (30 mg · kg⁻¹) dans la prévention des marqueurs physiologiques et pathologiques de l'atteinte pulmonaire aiguë. Six chiens prétraités avec du MPSS ont montré une augmentation aiguë de la pression de l'artère pulmonaire (PAP) en moins d'une minute après la BCA (17,8 ± 7,3 mmHg), augmentation qui était semblable à celle observée chez sept chiens non traités (control) (18,6 ± 12,6). L'augmentation de la résistance vasculaire pulmonaire (PVR) était semblable dans les deux groupes (455 ± 323 et 319 ± 137 dyne · sec · cm⁻⁵) et la diminution de la PaO₂ dans le groupe control était de 18,3 ± 6,4 mmHg par opposition à 12,4 ± 7,7 mmHg dans le groupe MPSS à cinq minutes après la BCA. Les concentrations artérielles circulantes de thromboxane B₂ (TxB₂) ainsi que les concentrations plasmatiques veineuses mélangées ont augmenté en dedans de une minute après la BCA dans les deux groupes, sans augmentation du gradient transpulmonaire. La concentration plasmatique artérielle de 6-keto prostaglandine F_{1α} (6-keto PGF_{1α}) a augmenté dans les deux groupes (0,91 ± 0,29 ng · ml⁻¹ et 0,87 ± 0,43 ng · ml⁻¹) une minute après la BCA. La concentration plasmatique veineuse mélangée de 6-keto PGF_{1α} a également augmenté, mais un gradient transpulmonaire significatif de 6-keto PGF_{1α} a été mis en évidence. Nous concluons que l'embolie aiguë graisseuse et de moëlle après une BCA est associée à des niveaux augmentés de TxB₂ et de 6-keto PGF_{1α}. La source de 6-keto PGF_{1α} suite à une embolie graisseuse pulmonaire aiguë est pulmonaire et ni les changements hémodynamiques aigus, ni la réponse prostanoid ne sont inhibés par la MPSS.

Key words

COMPLICATIONS: embolism, haemodynamic;

EMBOLISM: fat;

HORMONES: prostaglandins;

SURGERY: orthopaedic.

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Methylprednisolone sodium succinate (MPSS) has been shown to be effective therapy for fat embolism associated with post-traumatic lung injury,^{1,2} one cause of the adult respiratory distress syndrome (ARDS). In spite of controversy regarding the use of steroids,³⁻⁶ recent reviewers⁷ still suggest that MPSS may be efficacious in ameliorating the acute effects of fat embolism when given early after multiple long bone fractures. Proposed mechanisms by which high-dose steroids may reduce acute lung injury after fat embolism include: inhibition of phospholipase A₂,⁸ and acting as a free radical scavenger.⁹ Evaluation of the acute pulmonary physiological and pathological derangements after fat embolism has been hampered by the lack of a relevant animal model of the disease process.

Acute pulmonary fat embolism is produced predictably by a bilateral cemented arthroplasty (BCA) procedure performed in anaesthetized mongrel dogs.^{10,11} This acute lung injury is characterized by elevated pulmonary artery pressures (PAP), with increased pulmonary vascular resistance (PVR), and reductions in systemic arterial oxygen tension (PaO₂) with increases in intrapulmonary venous admixture ($\dot{Q}_{va}/\dot{Q}_l$).^{4,5} Infusion of fat and marrow from donors^{12,13} produced marked increases in pulmonary lymph and arterial concentrations of 6-keto prostaglandin F_{1 α} (6-keto PGF_{1 α}) in recipient animals. These stable by-products of arachidonic acid metabolism may mediate pulmonary hypertension and lung permeability changes in the fat embolism syndrome (FES).^{12,13}

This study was designed to determine whether a prostanoid response to fat and marrow embolism after BCA was present *in vivo*. This study also evaluated the efficacy of MPSS in modulating the acute increase in 6-keto PGF_{1 α} and TxB₂. Criteria for assessing the efficacy of MPSS in this study were the prevention or reduction of these pathological and physiological hallmarks of acute lung injury.

Methods

This study was approved by the local animal care committee and all dogs were treated in accordance with the regulations of the Canadian Council on Animal Care. Thirteen mongrel dogs weighing 29.0 ± 5.5 kg were anaesthetized using pentobarbitone $30 \text{ mg} \cdot \text{kg}^{-1}$ *iv*, followed by a continuous infusion ($0.5 \text{ mg} \cdot \text{kg} \cdot \text{hr}^{-1}$) of pentobarbitone as required to maintain anaesthesia. The trachea was intubated in each animal. The lungs were mechanically ventilated with a non-rebreathing circuit using room air. Ventilation was adjusted prior to surgery to keep arterial blood PCO₂ (PaCO₂) at 35–40 mmHg. Animals were paralyzed using pancuronium bromide ($0.2 \text{ mg} \cdot \text{kg}^{-1}$) and lactated Ringer's solution was infused at a rate of $10 \text{ ml} \cdot \text{kg} \cdot \text{hr}^{-1}$ *iv* to maintain circulating intravascular volume during the procedure.

The thirteen dogs were randomly divided into two groups. In the control group ($n = 7$), a bilateral cemented arthroplasty (BCA) procedure was performed as described in previous studies.^{10,11} In the control group, animals were given an infusion of 200 ml of lactated Ringer's solution during a 20-min period before reaming of both intramedullary canals. In the MPSS pre-treated group ($n = 6$), each dog was given an infusion of methylprednisolone sodium succinate ($30 \text{ mg} \cdot \text{kg}^{-1}$) *iv* in 200 ml of lactated Ringer's solution over 20 min prior to drilling and reaming of both intramedullary cavities. A BCA procedure was then performed in all animals.

In both groups the surgical technique, the physiological and the pathological measurements used, were standardized as described below. The plasma assays and quantitative morphometric measurements were performed without prior knowledge of animal pre-treatment with MPSS.

Surgical procedure

Surgical exposure of both distal femoral condyles preceded any physiological measurements. Both femoral condyles were then drilled and reamed retrogradely to a depth of 10 cm using successively larger reamers until a size 9 reamer was readily inserted. After reaming, all physiological measurements and assays were repeated. Low-viscosity bone cement was mixed according to the standard directions and injected into both intramedullary canals simultaneously under manual pressure. Solid contoured metal prostheses were then hammered into both intramedullary cavities to simulate prosthesis insertion of the clinical procedure. All measurements were repeated at predetermined intervals after BCA as specified.

Physiological measurements

In all dogs, a #7 Fr. triple lumen balloon-tipped pulmonary artery catheter was inserted into the left femoral vein and positioned in the pulmonary artery to measure continuously pulmonary artery pressure (PAP) and right atrial pressure (RAP). Thermodilution cardiac output ($\dot{Q}$) was measured in triplicate and a mean of three determinations at each measurement period was used as representative of the $\dot{Q}$ at each measurement time. The left femoral artery was cannulated to measure systemic arterial blood pressure (BP) and a Cordis #7 Fr. pigtail catheter was inserted into the left carotid artery and advanced through the left ventricle to the left atrium to monitor left atrial pressure (LAP) continuously. Pulmonary vascular resistance (PVR) was calculated at each measurement period. All pressures were continuously recorded on a Gould ES 1000 recorder.

Samples of arterial and mixed venous blood (from PA catheter) were obtained for blood gas analysis and all values were corrected for body temperature. Using a

one-way valve in the anaesthetic circuit, mixed expired gas was collected and analyzed for the concentration of oxygen and carbon dioxide. Intrapulmonary venous admixture ($\dot{Q}_{va}/\dot{Q}_l$) and physiological deadspace to tidal volume ratio (V_D/V_T) were calculated¹⁴ using these data.

Baseline haemodynamic and gas exchange measurements were made after drilling and reaming of the femoral intramedullary canals. The post-reaming measurements served as baseline values before BCA was performed. The post-reaming value was used as baseline because it is taken after the blood loss of reaming and was close in time to the measurements made after cement and prosthesis insertion. All measurements were repeated at 5, 15, 30 and 60 min after cement and prosthesis insertion. Haemodynamic pressure measurements and an arterial blood sample were also taken one minute after BCA to assess the immediate effect on cardiopulmonary function before the five-minute measurement. The change from post-reaming baseline value was used to compare groups.

Plasma assays

We sampled blood for measurement of the arachidonic acid metabolites, thromboxane B_2 (TxB_2) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$). Blood samples were drawn simultaneously from the pulmonary artery and from the descending thoracic aorta at the following time intervals: prior to reaming, immediately after reaming, one, five, fifteen, thirty and sixty minutes following BCA.

Each blood sample (9 ml) was collected in a plastic syringe containing 1 ml of an indomethacin/anticoagulant (EDTA) solution. This solution was prepared by diluting the stock indomethacin (0.04 M indomethacin in ethanol) 1:20 in an EDTA anti-coagulant which was prepared by dissolving 2 g disodium EDTA and 0.8 g of sodium chloride in 100 ml of water. Indomethacin was added to inhibit any further production of arachidonic acid metabolites *in vitro* after sampling. The pH was adjusted to 7.4 with a sodium hydroxide solution. Each blood sample was then mixed and immediately transferred to a plastic centrifuge tube, centrifuged and the plasma collected. The plasma was then transferred to polypropylene tubes and frozen immediately at -20°C for storage.

Thromboxane B_2 (TxB_2), the stable metabolite of thromboxane A_2 and 6-keto $PGF_{1\alpha}$, the stable metabolite of prostacyclin, were determined by radioimmunoassay (RIA) kits using labelled I^{125} tracers from New England Nuclear (DuPont). All plasma samples and standards were analyzed in duplicate. Samples were measured and standard curves were constructed. The gamma counter was LKB Model 1277 (Finland). The intra- and inter-assay coefficients of variation were respectively 7.6% and 6.1% for TxB_2 measurements at $0.1\text{ ng}\cdot\text{ml}^{-1}$ and 4.0%

TABLE I Baseline measurements (mean $\pm$ 1 SD) of haemodynamic, gas exchange and prostanoid measurements in each group, made after drilling and reaming both femoral intramedullary canals

	Control (n = 7)	MPSS (n = 6)
BP (mmHg)	148 $\pm$ 5	138 $\pm$ 17
PAP (mmHg)	14.4 $\pm$ 3.5	18.6 $\pm$ 6.8
LAP (mmHg)	4.3 $\pm$ 2.8	3.6 $\pm$ 7.5
RAP (mmHg)	2.9 $\pm$ 1.2	4.3 $\pm$ 2.0
$\dot{Q}$ ($\text{L}\cdot\text{min}^{-1}$)	3.2 $\pm$ 0.8	4.0 $\pm$ 0.6
PVR ($\text{dyne}\cdot\text{sec}\cdot\text{cm}^{-5}$)	260 $\pm$ 86	298 $\pm$ 112
SVR ($\text{dyne}\cdot\text{sec}\cdot\text{cm}^{-5}$)	3856 $\pm$ 1193	2766 $\pm$ 559
PaO_2 (mmHg)	96.0 $\pm$ 8.5	87.2 $\pm$ 17.7
$\dot{Q}_{va}/\dot{Q}_l$ (%)	1.0 $\pm$ 1.9	8.2 $\pm$ 8.2
PaCO_2 (mmHg)	36.3 $\pm$ 1.4	38.5 $\pm$ 4.5
V_D/V_T (%)	52 $\pm$ 6	50 $\pm$ 3
art. 6-keto $PGF_{1\alpha}$ ($\text{ng}\cdot\text{ml}^{-1}$)	0.31 $\pm$ 0.12	0.28 $\pm$ 0.14
mix. ven. 6-keto $PGF_{1\alpha}$ ($\text{ng}\cdot\text{ml}^{-1}$)	0.36 $\pm$ 0.12	0.30 $\pm$ 0.18
art. TxB_2 ($\text{ng}\cdot\text{ml}^{-1}$)	0.78 $\pm$ 0.59	0.90 $\pm$ 0.27
mix. ven. TxB_2 ($\text{ng}\cdot\text{ml}^{-1}$)	0.88 $\pm$ 0.41	1.01 $\pm$ 0.44

and 9.4% for 6-keto $PGF_{1\alpha}$ at $0.1\text{ ng}\cdot\text{ml}^{-1}$. Results from duplicate samples showed good reproducibility ($<10\%$). The lowest level of detection using these methods was $0.0005\text{ ng}\cdot\text{ml}^{-1}$ and $0.01\text{ ng}\cdot\text{ml}^{-1}$ for TxB_2 and 6-keto $PGF_{1\alpha}$ respectively.

Morphometry of lung tissue

Sixty minutes after cement and prosthesis insertion, the anaesthetized dogs were killed by injecting KCl through the left atrial cannula. A post-mortem thoracotomy was performed and the pulmonary vessels ligated. The lungs were removed en bloc and fixed in inflation with 10% buffered formalin at a pressure of 25 cm of fixative. After fixation for 72 hr, the lungs were sectioned in the mid-sagittal plane and three stratified random blocks of known size were taken from the mid-sagittal slice of each lung. These samples were post-fixed in Fleming's solution for 72 hr, washed in running water for 24 hr, then processed for histological examination in the usual manner and cut at $5\text{ }\mu\text{m}$ thickness.

The sections were then evaluated using a TV-based image analysis system (BQ Meg IV, R and M Biometrics, Nashville TN) with a Leitz Dialux 22 microscope attached to a Sony CCD video camera connected to an IBM AT compatible microcomputer. For each animal, 12 stratified random fields (each 1 mm^2) from the six lung samples were examined for a total area of 72 mm^2 per animal. The measurements were made at a final optical magnification of $245\times$. The gray scale image was converted to a binary (black and white) image by manual selection of the threshold value which best delineated the emboli from background. The area and diameter was calculated for

TABLE II Change (Δ) in haemodynamic measurements from post-reaming baseline values (mean $\pm$ 1 SD) to each measurement in both groups. *Denotes a significant change from post-reaming baseline value and # denotes a significant difference between groups in the change from baseline value

		1 min	5 min	15 min	30 min	60 min
Δ BP	Control	$-25.6 \pm 15.6^*$	-5.0 ± 14.0	-1.1 ± 2.9	-4.1 ± 11.6	-0.7 ± 15.5
	MPSS	$-23.9 \pm 17.8^*$	4.7 ± 20.0	4.7 ± 19.6	5.3 ± 23.1	3.6 ± 24.7
Δ PAP	Control	$18.6 \pm 12.6^*$	$15.9 \pm 11.1^*$	$11.6 \pm 6.7^*$	$10.8 \pm 6.1^*$	$11.0 \pm 4.9^*$
	MPSS	$17.8 \pm 7.3^*$	$13.0 \pm 5.3^*$	$11.3 \pm 2.4^*$	$10.8 \pm 3.3^*$	$9.8 \pm 4.0^*$
Δ LAP	Control	1.0 ± 2.0	0.9 ± 1.8	0.7 ± 1.6	-0.3 ± 3.0	0.2 ± 1.7
	MPSS	0.1 ± 2.0	0.8 ± 1.4	1.6 ± 2.7	1.7 ± 4.5	1.0 ± 4.3
Δ RAP	Control	0.9 ± 1.5	0.8 ± 1.1	0.8 ± 1.1	0.6 ± 1.0	0.9 ± 1.2
	MPSS	1.2 ± 1.3	0.7 ± 1.2	0.2 ± 1.7	0.3 ± 1.5	0.5 ± 2.7
Δ $\dot{Q}$	Control		-0.3 ± 0.5	-0.2 ± 0.3	-0.3 ± 0.4	-0.3 ± 0.6
	MPSS		-0.1 ± 0.5	-0.3 ± 0.7	-0.4 ± 0.8	-0.5 ± 1.1
Δ PVR	Control		$455 \pm 323^*$	$342 \pm 234^*$	$388 \pm 302^*$	$345 \pm 178^*$
	MPSS		$319 \pm 137^*$	$251 \pm 112^*$	$253 \pm 141^*$	$274 \pm 143^*$
Δ SVR	Control		66 ± 1203	159 ± 535	398 ± 729	310 ± 1438
	MPSS		173 ± 452	288 ± 273	423 ± 241	572 ± 694

each embolus. The ratio of area occupied by fat emboli to total area of lung examined is equivalent to the volume proportion of lung tissue occupied by fat (Delesse's Principle). The number of fat emboli and volume proportion of lung tissue occupied by fat after BCA in each group were then compared.

Statistical analysis

Data are reported as mean values $\pm$ one standard deviation. Data were analyzed using the SAS[®] (Statistical Analysis System) statistical package. Unpaired *t* tests were used to compare the baseline measurements between groups. Other comparisons were made using the SAS general linear model repeated measures analysis of variance procedure. When a significant *F* ratio was present ($P < 0.05$) multiple comparisons within groups between baseline and other measurements were made using unadjusted pairwise comparisons as printed out by LSMEANS comparison procedure with adjustment made on the probability of rejection using Bonferonni's correction.¹⁵ If there was significant group and time interaction, multiple comparisons with baseline values within groups were determined using Dunnett's test. Comparisons between groups for measurements other than baseline were made using change from baseline. When a significant *F* ratio was present ($P < 0.05$) multiple comparisons between groups of change from baseline were made using unadjusted pairwise comparisons as printed out by LSMEANS comparison procedure with adjustment made on the probability of rejection using Bonferonni's correction.¹³

Results

There was no significant difference in the mean weight of dogs included in the control group (27.1 ± 5.4 kg) and the MPSS group (32.5 ± 6.2 kg). Tables I and II summarize data for haemodynamic and gas exchange measurements. There were no statistically significant differences between groups in the baseline measurements (Table I) made after reaming.

A significant decrease in the systemic arterial blood pressure (BP) was noted within one minute of BCA in both groups (Table II). The BP returned to baseline levels by the five-minute measurement in both groups (Table II). The decrease in BP from baseline to one minute after BCA was not significantly greater in the control (25.6 ± 15.6 mmHg) than in the MPSS group (23.9 ± 17.8 mmHg).

A significant increase in PAP and PVR was present after BCA in both groups (Table II). This elevated PAP and PVR persisted throughout the 60-min study period. The increases in both PAP and PVR were not significantly different between groups at any measurement time. No differences from baseline measurements were noted in $\dot{Q}$, RAP, LAP or calculated systemic vascular resistance (SVR) during the study period, either within any group or between groups (Table II).

A significant decrease in PaO_2 was noted in both groups within five minutes of BCA (Table II). This decrease in PaO_2 was accompanied by a significant increase in $\dot{Q}_{\text{va}}/\dot{Q}_{\text{t}}$ (Table II) in the control group and in the MPSS dogs. There was no significant difference between groups in the change in PaO_2 or $\dot{Q}_{\text{va}}/\dot{Q}_{\text{t}}$ at any

TABLE III Change (Δ) in gas exchange measurements from post-reaming baseline values (mean $\pm$ 1 SD) to each measurement in both groups. *Denotes a significant change from post-reaming baseline value

		1 min	5 min	15 min	30 min	60 min
Δ PaO ₂	Control	-10.8 $\pm$ 4.5*	-18.3 $\pm$ 6.4*	-17.5 $\pm$ 9.5*	-19.2 $\pm$ 9.2*	-18.4 $\pm$ 9.1*
	MPSS	-4.9 $\pm$ 7.5*	-12.4 $\pm$ 7.7*	-14.3 $\pm$ 8.2*	-14.3 $\pm$ 9.2*	-17.3 $\pm$ 8.2*
Δ $\dot{Q}_s/\dot{Q}_t$	Control		9.9 $\pm$ 5.4*	8.3 $\pm$ 5.0*	9.5 $\pm$ 6.7*	9.6 $\pm$ 7.1*
	MPSS		11.4 $\pm$ 11.8*	6.6 $\pm$ 6.6*	12.0 $\pm$ 14.2*	12.4 $\pm$ 13.0*
Δ PaCO ₂	Control	0.4 $\pm$ 1.3	-0.1 $\pm$ 1.1	-1.2 $\pm$ 1.2	-1.2 $\pm$ 1.9	-1.7 $\pm$ 2.6
	MPSS	0.8 $\pm$ 2.2	0.1 $\pm$ 2.7	0.5 $\pm$ 3.2	1.0 $\pm$ 4.9	1.7 $\pm$ 7.0
Δ V _D /V _T	Control		-0.5 $\pm$ 2.7	-3.2 $\pm$ 4.0	-2.4 $\pm$ 5.2	-4.2 $\pm$ 4.1
	MPSS		1.3 $\pm$ 3.8	2.0 $\pm$ 3.3	1.1 $\pm$ 3.6	3.0 $\pm$ 3.4

TABLE IV Change Δ in arterial and mixed venous concentration of 6-keto PGF_{1 α} and TxB₂ (mean $\pm$ 1 SD) from baseline value to each subsequent measurement in both groups. *Denotes a significant change from baseline value within this group

		1 min	5 min	15 min	30 min	60 min
Δ 6-keto PGFa	Control	0.91 $\pm$ 0.29*	0.45 $\pm$ 0.25	0.09 $\pm$ 0.18	0.26 $\pm$ 0.46	0.16 $\pm$ 0.40
	MPSS	0.87 $\pm$ 0.43*	0.24 $\pm$ 0.16	0.01 $\pm$ 0.08	-0.01 $\pm$ 0.08	0.00 $\pm$ 0.11
Δ 6-keto PGFv	Control	0.46 $\pm$ 0.16*	0.31 $\pm$ 0.12*	0.14 $\pm$ 0.12	0.23 $\pm$ 0.39*	0.15 $\pm$ 0.35
	MPSS	0.48 $\pm$ 0.18*	0.22 $\pm$ 0.09*	0.04 $\pm$ 0.03	-0.01 $\pm$ 0.05	0.02 $\pm$ 0.10
Δ TxB _{2a}	Control	0.37 $\pm$ 0.40*	0.20 $\pm$ 0.55	0.14 $\pm$ 0.40	0.20 $\pm$ 0.37	0.02 $\pm$ 0.42
	MPSS	0.37 $\pm$ 0.35*	0.16 $\pm$ 0.41	0.03 $\pm$ 0.50	0.05 $\pm$ 0.42	-0.06 $\pm$ 0.29
Δ TxB _{2v}	Control	0.33 $\pm$ 0.28*	0.13 $\pm$ 0.21	0.23 $\pm$ 0.41	0.17 $\pm$ 0.30	0.02 $\pm$ 0.20
	MPSS	0.41 $\pm$ 0.35*	0.17 $\pm$ 0.32	0.04 $\pm$ 0.34	0.02 $\pm$ 0.45	0.02 $\pm$ 0.24

measurement time. The value of V_D/V_T did not change significantly from baseline in either group throughout the study period (Table III), and no significant differences were noted between groups.

The baseline arterial concentration of TxB₂ after reaming of the intramedullary canals was not different between the control (0.78 $\pm$ 0.59 ng $\cdot$ ml⁻¹) and the MPSS (0.90 $\pm$ 0.27 ng $\cdot$ ml⁻¹) groups. Both groups demonstrated significant increases in the arterial and mixed venous concentration of TxB₂ at the one minute measurement (Table IV). No significant change from the post-reaming baseline value of the transpulmonary (arterial-mixed venous) gradient of TxB₂ was detected at any time in either group (Figure 1).

The arterial and mixed venous plasma concentrations of 6-keto PGF_{1 α} in both groups increased significantly from baseline level within one minute of BCA (Table IV). No difference was detected between the control and the MPSS groups at any time period in the transpulmonary gradient of 6-keto PGF_{1 α} (Figure 2). A significant increase in the transpulmonary gradient of 6 keto PGF_{1 α} at the one-minute measurement was noted in the control (0.45 $\pm$ 0.26 μ g $\cdot$ ml⁻¹) and in the MPSS (0.38 $\pm$ 0.32 μ g $\cdot$ ml⁻¹) groups, returning to baseline values within five minutes of BCA (Figure 2).

Quantitative morphometry of the lung showed that the proportion of lung volume occluded by fat emboli was 1.31 $\pm$ 0.29% in the control group and 1.30 $\pm$ 0.54% in MPSS group. There was no significant difference between groups. The size of the pulmonary vessels occluded by fat ranged from 7.6 μ to 14.2 μ in all dogs and no significant difference was found between groups in the size distribution of pulmonary fat emboli in the lung.

Discussion

Fat and marrow embolism causes an acute lung injury both in experimental animals¹⁰⁻¹³ and in clinical practice.^{1,2} This study reports that products of arachidonic acid metabolism with potent biological effects are produced *in vivo* during marrow-induced lung injury after BCA. These prostanoids are known to be produced by endothelial cells and inflammatory cells of the lung as well as platelets and may act as mediators and/or markers of acute lung injury.⁷ The time sequence of prostanoid changes (Table IV; Figures 1, 2) relative to the pulmonary hypertension after BCA (Table I) are very different. The early, transient prostanoid changes are normalized while the increase in PAP and PVR is prolonged, suggesting that the pulmonary hypertension is not solely caused by the release of the vasoconstrictor TxA₂.

FIGURE 1 The change (Δ) in transpulmonary gradient (arterial-mixed venous concentration) of TxB_2 from the post-reaming baseline to all subsequent measurements.

FIGURE 2 The change (Δ) in transpulmonary gradient (arterial-mixed venous concentrations) of 6-keto $\text{PGF}_{1\alpha}$ from post-reaming baseline to all subsequent measurements.

In this study, an equivalent "load" of pulmonary fat emboli was found in the lungs of both groups. Siegwert *et al.*¹⁷ using quantitative morphometric techniques determined that eight per cent of the lung volume was composed of vessels. Using his data,¹⁷ we estimated that in the mid-sagittal lung slices examined, approximately 16.4% (1.31/8) and 17.4% (1.39/8) of vessels were occluded in the control and MPSS groups respectively. Since over 50% of the pulmonary vasculature must be mechanically occluded before PAP or PVR increases,^{18,19} we conclude that either (i) vasoconstricting mediators are released augmenting the pulmonary hypertension associated with mechanical occlusion or (ii) feeding vessels in the lung are occluded with fat, thus mechanically obstructing larger proportions of the pulmonary vasculature than estimated by quantitative morphometric techniques.

Clinical studies of effective attenuation of acute physiological derangements in post-traumatic fat embolism have been hampered by associated factors such as the presence of aspiration pneumonitis, lung contusion and haemo-pneumothorax. The BCA model¹⁰ permits an objective evaluation of the early physiological changes after fat embolism, the impact of pharmacological intervention in the FES independent of these associated factors and quantification of the degree of fat embolism which is not possible in clinical studies. Before initiating this study, we chose four markers of acute lung injury to study the effect of MPSS. These markers were: increased PVR, hypoxaemia, increased $\dot{Q}_{va}/\dot{Q}_t$ and the generation of prostanoid products of arachidonic acid metabolism. The degree of fat embolism in these dogs was not sufficient to cause severe hypoxaemia (Table II); however, it did cause an increase in $\dot{Q}_{va}/\dot{Q}_t$ with significant pulmonary hyper-

tension, elevated circulating concentrations of TxB_2 (Figure 1, Table IV) and increased pulmonary production of 6 keto $\text{PGF}_{1\alpha}$, as evidenced by the transpulmonary gradient (Figure 2 Table IV). Pre-treatment with MPSS ($30 \text{ mg} \cdot \text{kg}^{-1}$) did not attenuate the acute changes in any of these markers of acute lung injury after fat embolism.

The controversy regarding the use of MPSS in septic shock and ARDS has been largely resolved and few investigators³⁻⁶ believe MPSS ameliorates this lung damage. However, reviews⁷ still advocate the use of steroids specifically after long bone fracture to reduce lung injury from FES. Within the 60 min time frame of our study, these data suggest that there was no beneficial effect of MPSS pre-treatment on the acute physiological abnormalities after fat embolism. However, we cannot exclude a beneficial effect that might accrue later in the disease process from MPSS. Similarly, after air embolism¹⁶ methylprednisolone given before the insult did not alter the acute haemodynamic changes but later did ameliorate the degree of lung injury which is dependent on leukocytes.

The degree of pulmonary hypertension (Table I) in this study after BCA is similar to that documented by others where animals (sheep and goats) were infused with marrow from donors.^{12,13} Arachidonic acid metabolites have been implicated as mediators of acute pulmonary hypertension and increased lung permeability after marrow infusion.¹² TxA_2 , the precursor of TxB_2 , is a potent vasoconstrictor and prostacyclin, the precursor of 6-keto $\text{PGF}_{1\alpha}$ is a known vasodilator. Hence, these prostanoids could be active *in vivo* products of acute lung injury after fat and marrow embolism during cemented arthroplasty. Our data support the suggestion that the lung is the source of prostacyclin after embolic injuries since the transpul-

monary 6-keto $\text{PGF}_{1\alpha}$ gradient increased significantly (Figure 2).

Unlike acute lung injury after endotoxin²⁰ our studies showed that the lung is not the source of TxB_2 . One likely source is the reamed contents of the intramedullary cavity and/or platelets. Endotoxin models²¹ of ARDS have suggested that cyclooxygenase enzyme inhibition reduces the rise in both 6-keto $\text{PGF}_{1\alpha}$ and TxB_2 and inhibition of this enzyme attenuates the early increase in PAP. This suggested that TxA_2 may partially mediate the pulmonary hypertension accompanying ARDS. TxA_2 receptor antagonists have recently been shown to attenuate the acute pulmonary hypertension associated with protamine-heparin complex formation.²² The relevance of these studies to lung injury induced by particulate fat and marrow and other causes of ARDS remains uncertain. The role of leukocytes, complement activation and mediator release in acute lung injury may vary, depending on the initiating insult.

The degree of lung injury in this model may be insufficient to cause large permeability changes detectable by measuring $\dot{Q}_{va}/\dot{Q}_t$. As well, an increase in lung permeability with large increases in $\dot{Q}_{va}/\dot{Q}_t$ may not have been evident in these healthy dogs at such low LAP values (Table I, II) after BCA. If the hydrostatic microvascular pressure in the lung had been increased by augmenting intravascular volume, we may have evaluated the effect of MPSS pre-treatment on permeability and gas exchange more effectively. Permeability increases may require a more marked endothelial cell injury, a more sustained microvascular pressure gradient or a more sensitive measurement than $\dot{Q}_{va}/\dot{Q}_t$ for detection.

The release of prostacyclin from the pulmonary endothelium is a known response of the lung to embolic injuries.^{12,13} Clinicians have noted transient systemic hypotension and pulmonary hypertension after cement and prosthesis insertion during cemented joint replacements for many years.²³ These changes have been attributed to: toxic effects of absorbed methylmethacrylate monomer;²⁴ neurogenic reflexes;²⁵ the release of thromboplastic products;²⁶ and to mechanical haemodynamic sequelae of air²⁷ and fat^{10,26} emboli. The presence of pulmonary hypertension and systemic hypotension, as noted in both the control and MPSS groups (Table I), has been reported in this model.^{10,11} The release of prostacyclin from injured lung and TxA_2 from marrow or platelets may contribute to the physiologic changes noted.

In conclusion, our studies of the acute haemodynamic and gas exchange response to fat and marrow embolism after BCA did not demonstrate that pre-treatment with MPSS attenuated either the haemodynamic or prostanoid response to cemented arthroplasty. This study does show that products of arachidonic acid metabolism, both 6-keto

$\text{PGF}_{1\alpha}$ and TxB_2 , are released during cemented arthroplasty.

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