Aetiology of pulmonary dysfunction in total hip replacement operations

The influence of nifedipine on the factors involved

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Summary

Patients undergoing total hip replacement surgery who developed pulmonary dysfunction (PD) demonstrated evidence of complement activation and increased thromboxane A_2 (TXA_2) synthesis. In a double-blind study nifedipine (Adalat; Bayer-Miles) was shown to inhibit complement activation and TXA_2 synthesis and thus appears to offer protection against PD.

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It is well established that activation of the complement system resulting in activation of leucocytes, and subsequent pulmonary capillary endothelial damage and leakage of serum and red blood cells into the interstitial tissue and alveoli, are integral components of the adult respiratory distress syndrome (ARDS).[1] Uncertainty exists about the aetiology of the vascular changes present in ARDS, but prostaglandins, notably thromboxane A_2 (TXA_2), seem to play an important role.[2]

Fein *et al.*[3] stated that pulmonary dysfunction (PD) is just a milder form of ARDS, and depending on the trigger mechanism may progress to ARDS. Both terms will be used as appropriate or in accordance with the terminology used in our references in this report.

The aim of this study was to establish the role of the complement system in the development of PD in patients undergoing total hip replacement under general anaesthesia and the interrelationships between complement activation, TXA_2 and prostacyclin (PGI_2) levels in this condition. Finally, in a double-blind study the effect of nifedipine (Adalat; Bayer-Miles) administration on the incidence of PD was investigated.

Patients and methods

The protocol was approved by the ethics committee of the University of Stellenbosch Medical School and informed consent was obtained from all patients involved, who were scheduled for hip replacement surgery. No prostaglandin antagonists or steroids were allowed during the week preceding the operation. Patients were randomly allocated to the treatment or placebo group in a double-blind manner. Twenty-one patients (18 women and 3 men, mean age 74 years, range 66 - 84) years were entered into the study. The 11 patients in the treatment group received oral nifedipine 10 mg 8 hourly for 2 days before surgery, on the day of operation and on the first postoperative day; 10 patients served as controls. Both groups were given a general anaesthetic with similar agents and were ventilated with a fractional oxygen concentration (Fio_2) of 0,4.[4]

Monitors. Arterial pressure was continuously monitored via a 20 G radial artery catheter, an AE 840 pressure transducer and a Simonsen and Weel oscilloscope with digital display. Central venous pressure was monitored with a water manometer. Expiratory carbon dioxide was monitored using a Godart infant capnograph. Arterial carbon dioxide tension ($Paco_2$), arterial partial pressure of oxygen (Pao_2) and pH were determined with a Gas Check AVL apparatus at the same time that other measurements were performed. ECG, oesophageal temperature and Fio_2 were also monitored continuously. During the trial period all parameters were within normal ranges.

Frequency of sampling

Blood samples were withdrawn at set measurement times: (*i*) just before induction of anaesthesia; (*ii*) 30 minutes after stable anaesthesia was established; (*iii*) 5 minutes after femoral prosthesis placement; (*iv*) at the end of the operation; and (*v*) about 24 hours after the operation.

Sampling

Thromboxane B_2 (TXB_2 — the stable metabolite of TXA_2), 6-ketoprostaglandin (PG) $F_1\alpha$ — the stable metabolite of PGI_2), activated complement 5 (C5a), β-thromboglobulin (β-TG), a full blood count, coagulation profile and blood gases were determined.

Preparation of tubes for TXB_2, 6-ketoPGF$_1\alpha$, and β-TG specimens

Stock solutions were prepared as follows: 10 g/dl ethylenediaminetetra-acetic acid (EDTA) and 540 g/dl theophylline were dissolved in distilled water and the pH adjusted to 7,0. Aspirin 120 mg was added to 1 ml of methyl alcohol.

On the day of sampling 40 μl of aspirin solution was added to 200 μl theophylline-EDTA solution in precooled (4°C) polypropylene collecting tubes. Five ml of radial arterial blood was collected into each tube and mixed. The tubes were immediately put in crushed ice and transported to the laboratory. Tubes were centrifuged at 4°C and 1 500 x g for 10 minutes and the upper portion of the plasma layer was removed and the centrifugation and sampling repeated.

The remainder of the platelet-poor plasma was acidified with 1M hydrochloric acid (1 volume of sample to 0,5 volume of acid) and extracted into 10 volumes of diethyl-ether in polypropylene tubes. The organic phase was separated, evaporated to dryness under nitrogen (37°C) and the residue dissolved in assay buffer (0,25 volumes); (3H) — $PGF_2\alpha$ (Amersham International), which does not crossreact appreciably with the antiserum used to determine TXB_2 (< 1%) and 6-ketoPGF$_1\alpha$ (2,2%), was used to monitor

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extraction recovery (50 µl). This was greater than 85% in all cases. The remainder of the specimen was stored in aliquots at −20°C until assay of TXB_2 and 6-ketoPGF$_1\alpha$.

Assay

For TXB_2 and 6-ketoPGF$_1\alpha$ determinations, in-house radio-immunoassay kits were used. Antiserum and standard were obtained from Seragen Inc. and titrated isotopes from New England Nuclear Inc. The buffer used was 0,01M phosphate (pH 7,4) containing 1,0% bovine serum albumin, and separation of bound from free tracer was achieved using 1% 1 : 1 dextran-coated charcoal in distilled water. Sample size was 100 µl and results were corrected using homologous nonspecific binding values (no antiserum: 5 -7% of total counts) and an internal standard incorporated into the radio-immunoassay procedure. Intra-sample coefficient of variation was less than 7%.

For β-TG and C5a in EDTA plasma estimations commercial radio-immunoassay kits were used.

Criteria for diagnosing PD

All patients were examined twice daily for 1 week. The usual pattern in those affected was that the patient appeared well at one postoperative visit, but at the next visit complained of shortness of breath and 'feeling out of sorts'. This usually occurred at the 24- or 36-hour postoperative visits, at a stage at which post-anaesthetic anoxia in surgery on extremities is not usually associated with reduced Pao_2.[5]

Examination of the affected patients revealed the following: (*i*) tachypnoea (> 20/min); (*ii*) tachycardia (> 110/min); (*iii*) Pao_2 significantly lower than pre-operative level and expected for Fio_2 — patients with a diagnosis of PD had blood gases measured twice a day until they were at acceptable levels; and (*iv*) $Paco_2$ values at the lower limits of normal.

A raised temperature was not a feature, clinical examination of the lungs appeared normal while chest radiography, and flow-perfusion studies revealed no abnormalities.[4] These criteria correspond with Gomez's type I ARDS classification.[3] The diagnosis of PD could not have been influenced by C5a and TXB_2 results because they were only available 3 months later.

Results

Incidence of PD. In the treatment group 1 patient developed PD whereas 4 were affected in the control group. The incidence of PD in the control group was 37% as opposed to 9% in the treatment group.

Complement activity. In awake patients basal values of C5a ranged from 0 to 200 ng/ml. Because of the wide range, results were expressed as a percentage of the basal value (measurement time 1). Patients in the control group had a 5-fold increase in C5a levels over the nifedipine group at measurement time 3 (major trauma) (Fig. 1) ($P < 0,05$; unpaired Student's *t*-test). The 5 patients who developed PD demonstrated an 11-fold increase in C5a levels at measurement time 3 compared with the 16 patients who remained well (Fig. 2) ($P < 0,001$; unpaired Student's *t*-test). At measurement times 4 and 5 the differences between the groups were still statistically significant ($P < 0,05$; unpaired Student's *t*-test).

TXB_2. The range of basal values was 6 - 135 pg/ml which necessitated results being expressed as a percentage of those values. The 5 patients who developed PD had a statistically significant increase of TXB_2 ($P < 0,05$; unpaired Student's *t*-test) at measurement time 5 compared with the 16 patients who remained well (Fig. 3). TXB_2 levels in nifedipine-treated and control groups were not statistically different at any of the measurement times (Fig. 4). This apparent contradiction arose because of a very high TXB_2 level at measurement time 5 in the 1 patient in the nifedipine group who developed PD. We have demonstrated an excellent correlation ($r = 0,89$; $P < 0,001$) (Fig. 5) between β-TG and TXB_2 levels 24 hours after the hip replacement operation in 9 randomly selected patients. This suggests platelets as an important source of TXA_2 synthesis.

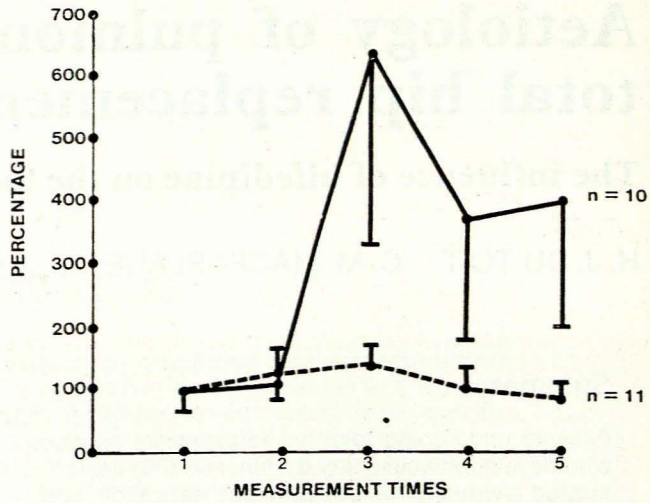

Fig. 1. C5a levels in the nifedipine and control groups expressed as a percentage change from initial value (mean + 1 SEM) (● - - - ● nifedipine; ●——● control).

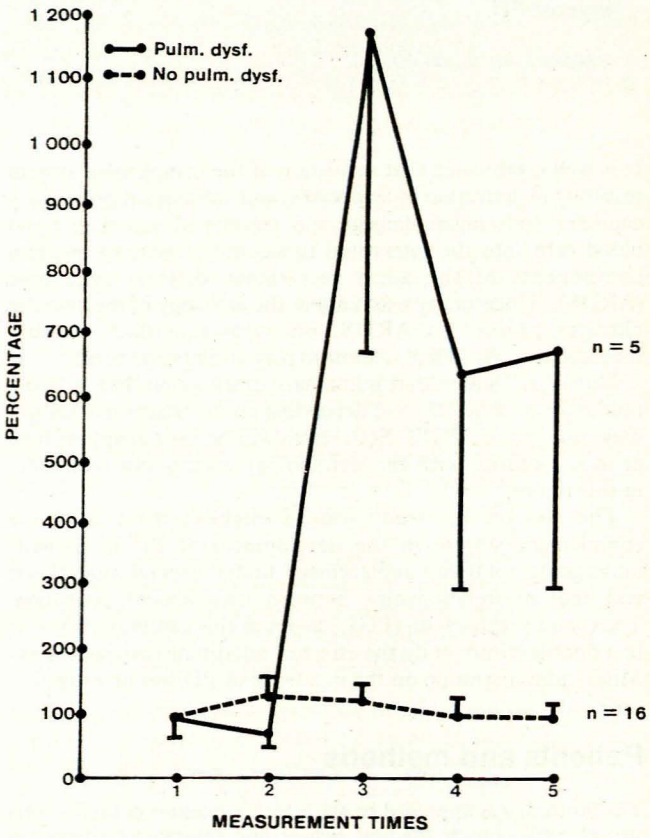

Fig. 2. C5a levels in patients who developed PD and those who did not, expressed as a percentage change from initial values (mean + 1 SEM).

6-ketoPGF$_1\alpha$. Basal values ranging between 36 pg/ml and 467 pg/ml necessitated expressing the results as a percentage of the initial values. There were no statistically significant differences at any measurement times between treated and control patients or between patients who developed PD and those who remained well.

Neutrophils. Patients who developed PD and unaffected ones had similar neutrophil counts at measurement time 4. Nifedipine-treated patients doubled their neutrophil counts half an hour after insertion of the prosthesis ($P < 0,05$; unpaired Student's *t*-test) in comparison with the control patients.

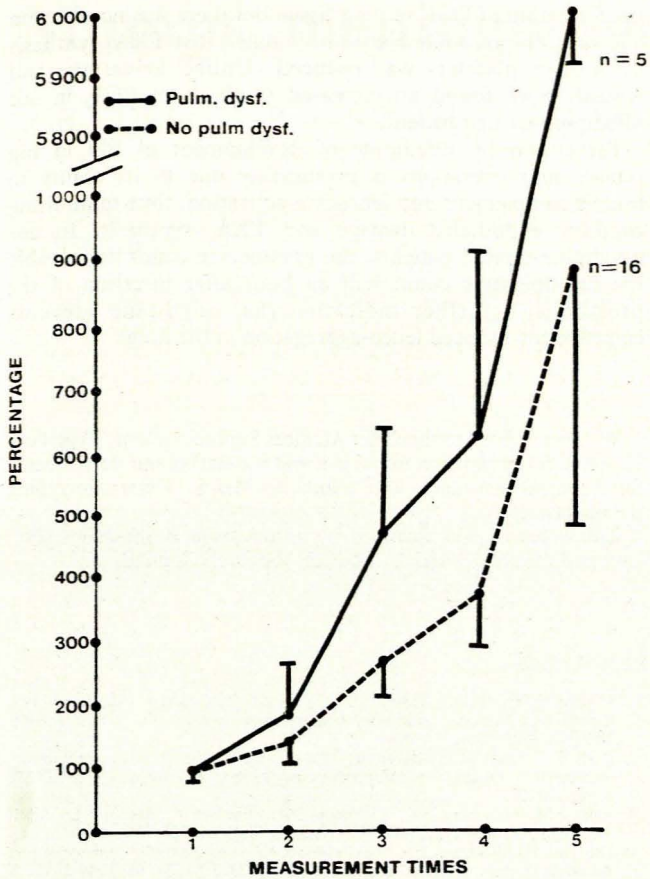

Fig. 3. TXB_2 levels in patients who developed PD and those who did not, expressed as a percentage change from initial value (mean + 1 SEM).

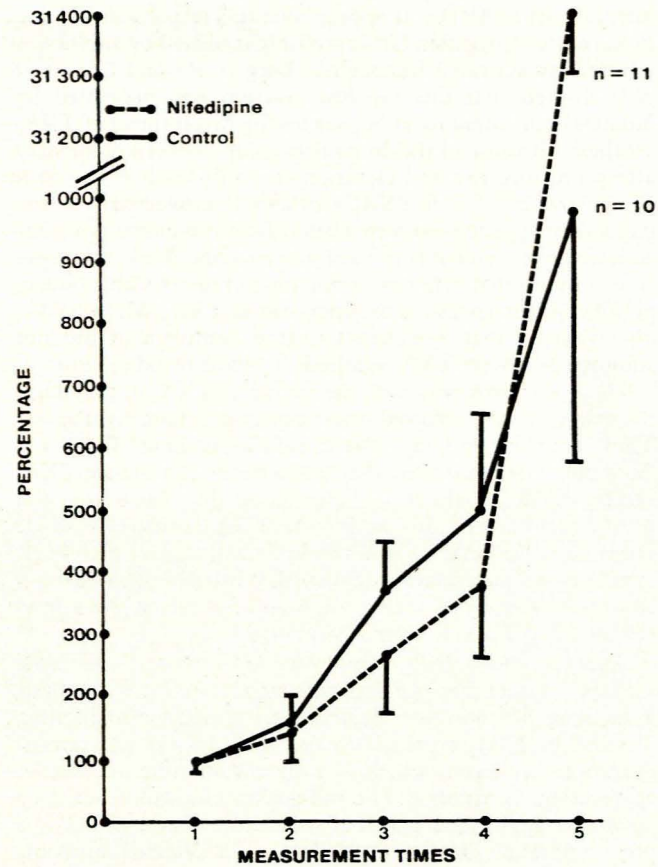

Fig. 4. TXB_2 levels in nifedipine and control groups expressed as a percentage change from initial values (mean + 1 SEM).

Monocytes. Patients in the control group who developed PD had a reduced monocyte count to just outside the 0,05 significance level in comparison with those who remained well about 30 minutes after major trauma (Mann-Whitney *U*-test).

Discussion

Many trigger mechanisms can activate the complement system, the most common being trauma, severe sepsis and acute pancreatitis, and inappropriate activation of this system may harm the host. Hammerschmidt *et al.*[6] have demonstrated that depending on the magnitude of the stimulus C5a activation is a factor in the genesis of PD or ARDS. Craddock *et al.*[7] have shown that changes in granulocyte counts are present in PD. In simulated haemodialysis experiments in sheep and rabbits, neutropenia and monocytopenia developed as a result of complement activation within 30 minutes of exposure to cellophane. This was associated with PD lasting 3 - 4 hours. In our patients who developed PD we could not demonstrate neutropenia but monocytopenia was present. When Craddock *et al.*'s[7] animals were sacrificed at 30 minutes the animal's pulmonary microvasculature was found to be occluded by white blood cells. These observations suggested that a plasma factor (probably the complement system) had promoted translocation of leucocytes from peripheral blood to form aggregates occluding the pulmonary microvasculature. Evidence has been provided that activated C5 or its desarginine derivative cause granulocytes to become nonspecifically adhesive and that they thus adhere to endothelium (hypermargination) and to each other (aggregation), and therefore could occlude the microvasculature.[1,8]

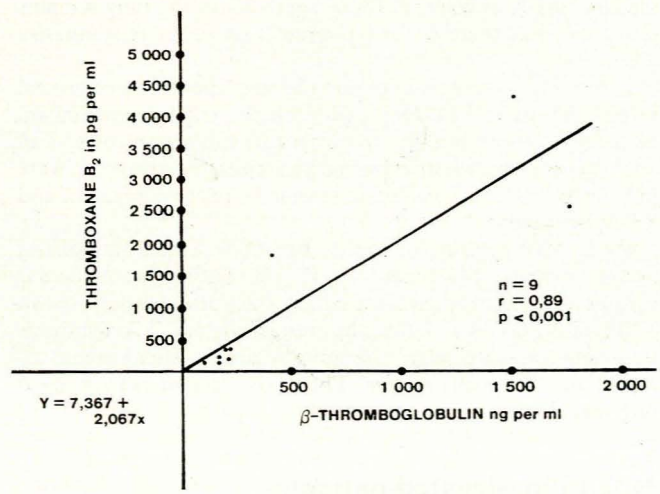

Fig. 5. Correlation coefficient between TXB_2 and β-TG levels 24 hours after total hip replacement.

These activated granulocytes lead to capillary endothelial damage and allow plasma and red cell leakage into interstitial tissues. Toxic oxygen radicals[9] originating from the action of 5-lipoxygenase on arachidonic acid[10] cause this endothelial damage. Complement activation can thus lead to 'leaky lungs'. In our control group it was demonstrated that total hip replacement (probably the trauma of operation) activates the complement system.

However, complement activation alone cannot explain the marked rise in pulmonary vascular resistance which is an

integral part of ARDS. It appears that this response is not due to leucocyte aggregation but is probably mediated by substances released by activated leucocytes.[2] Borg *et al.*[2] and Cooper *et al.*[11] showed that this vascular reaction was prevented by indomethacin administration, suggesting involvement of TXA_2 synthesis. Because of the dose relationship between pulmonary artery pressure rise and elevation of TXB_2 levels it has been demonstrated[11-13] that TXA_2 synthesis is associated with the pulmonary hypertension seen when plasma-containing zymosan-activated complement is infused into animals. While Goldstein *et al.*[14] found that zymosan stimulation of intact viable human peripheral leucocytes led to generation of TXA_2, McDonald *et al.*[13] showed that zymosan-activated complement did not stimulate leucocyte TXA_2 synthesis in whole blood *in vitro*.

There is controversy over the source of TXA_2 in the lungs. Svensson *et al.*[15] showed that guinea-pig lung synthesises TXA_2 actively, and in isolated guinea-pig lungs Piper and Samhoun[16] demonstrated that leukotrienes can induce TXA_2 synthesis. McDonald *et al.*[13] also found that sheep lung and pulmonary artery are active in TXA_2 synthesis while Ingerman-Wojenski *et al.*[17] have shown TXA_2 and PGI_2 synthesis by pulmonary endothelial cells. Although there is controversy over its exact origin, the evidence presented suggests that TXA_2 is synthesised in the lung.

TXA_2 is undoubtedly synthesised by platelets[15] yet some authors[13,18] state that platelets take no part in TXA_2 synthesis in the lung. We, however, feel that there is evidence implicating platelets in TXA_2 synthesis in the lung. In hip replacement operations, collagen, which is a potent stimulus of platelet aggregation, is exposed. The pulmonary circulation acts as a sieve and aggregated platelets translocate there. β-TG is a protein probably unique to platelets and is detected in plasma only if there has been platelet aggregation and release of TXA_2.[19] In the present study we demonstrated an excellent correlation between β-TG and TXB_2 levels 24 hours after operation in 9 randomly selected patients. Therefore, whether platelets are important in TXA_2 synthesis in the lung remains uncertain, but there is further proof that platelets synthesise TXA_2.

In summary, activation of complement appears to be crucial in the genesis of PD. Depending on the trigger mechanism, additional factors become important in subsidiary roles as in our patients, in whom trauma and collagen exposure were presumed to be responsible for major platelet aggregation and TXA_2 synthesis.

PGI_2-TXA_2 ratio. In the human body a delicate balance exists between TXA_2 and PGI_2, preventing intravascular thrombosis. According to our results the normal ratio between TXA_2 and PGI_2 is 1 : 2, but the stimulation of TXA_2 synthesis by major trauma changes the ratio to 20 : 2. Other authors[11,13] report similar results *in vivo*. The PGI_2 fraction only changed minimally.

Nifedipine-treated patients

In the 11 patients treated with nifedipine, C5a levels remained normal at all the measurement times. In the 10 control patients the C5a levels at measurement time 3 (major trauma) rose significantly higher than in the nifedipine-treated patients ($P < 0{,}05$; unpaired Student's *t*-test) (Fig. 1). Of the 11 patients treated with nifedipine, only 1 developed PD, but at measurement time 3 this patient had a C5a level only 0,7 times higher than the control value. It would thus appear that other mechanisms, among which is collagen, may also trigger massive platelet aggregation and TXA_2 synthesis. This patient had the highest TXB_2 level of all 24 hours after the operation.

There is controversy over the effect of nifedipine on TXA_2 and PGI_2 synthesis. In isolated rat aorta Srivastava and Awasthi[20] demonstrated that nifedipine significantly increased the formation of PGI_2 in lung tissue but there was no effect on TXA_2 synthesis, while Han *et al.*[21] stated that TXA_2 synthesis by human platelets was reduced. Unlike Srivastava and Awasthi[20] we found no increased synthesis of PGI_2 in our nifedipine-treated patients.

Prevention by nifedipine of development of PD in hip replacement operations is presumably due to its ability to inhibit complement and leucocyte activation, thus minimising capillary endothelial damage and TXA_2 synthesis. In our nifedipine-treated patients, the granulocyte count was double the pre-operative count half an hour after insertion of the prosthesis, a further indication that nifedipine prevents complement-induced leuco-aggregation in the lungs.

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