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Antagonisation of platelet activating factor – a new therapeutic concept for improvement of organ quality in lung preservation

S. W. Hirt, Th. Wahlers, M. Jurmann, L. Dammenhayn, R. Rohde, and A. Haverich

Division of Thoracic and Cardiovascular Surgery, Hannover Medical School, Hannover, Federal Republic of Germany

Abstract. The release of platelet activating factor (PAF) is thought to be one of the most important pathophysiological pathways in the development of ischemic lung injury. We investigated the use of a PAF antagonist (PAF-a) in a canine model in reducing PAF-mediated pulmonary dysfunction following lung preservation and transplantation. Twelve combined heterotopic heart and orthotopic left lung allotransplantations were performed after 6 h of cold ischemia. Following administration of prostacyclin (PGI_2), Euro-Collins solution (EC) was used for pulmonary artery flush in all donors, while in six animals the PAF-a, WEB 2170 BS, was administered to the donor (0.15 mg/kg for 30 min), to the storage solution (0.3 mg/kg) and to the recipient during reperfusion for a total of 6 h (0.3 mg/kg per h) EC/PAF-a). In all donors myocardial preservation was achieved using St. Thomas Hospital solution. Postoperatively, cardiorespiratory function was evaluated seperately for donor and recipient organs at an FiO_2 of 0.4 for a maximum of 12 h. The quality of lung preservation was assessed by means of postoperative oxygenation (pO_2), pulmonary artery pressure (PAP) and pulmonary vascular resistance index (PVRI). In the EC/PAF-a group, pO_2 of the donor lung was significantly elevated ($P < 0.01$) and PVRI was significantly lower ($P < 0.05$) when compared to the EC group, while PAP showed no significant differences between both groups and throughout the entire postoperative course. We concluded that a significant improvement in the current clinical standard for lung preservation could be obtained by the application of WEB 2170 BS in combination with EC flush as demonstrated by improved oxygenation and lower PVRI of the transplantated organs.

Key words: PAF-antagonist – Prostacyclin – Euro-Collins solution – Heart and lung transplantation

Offprint requests to: Stephan W. Hirt, M.D., Division of Thoracic and Cardiovascular Surgery, Hannover Medical School, Konstanty-Gutschow-Strasse 8, D-3000 Hannover, FRG

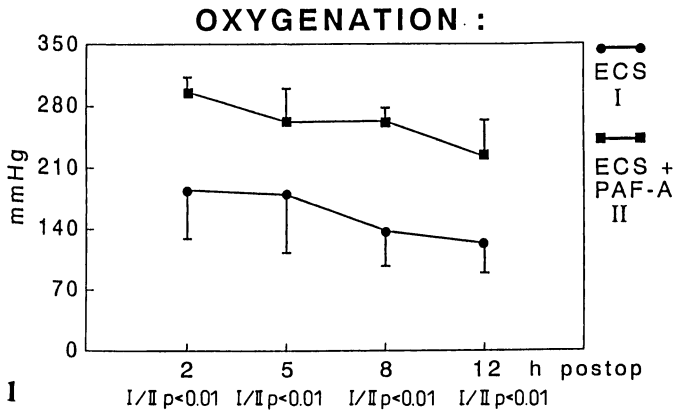
Initially, attempts at lung transplantation failed in the early postoperative period due to the poor preservation quality of the transplanted organs [1]. Several methods for distant organ procurement have been succesfully established [2–5]. Currently, pulmonary artery flush perfusion with Euro-Collins solution (EC) in combination with prostacyclin (PGI_2) pretreatment is most frequently used for lung preservation [6–9] and acceptable results are achieved clinically within ischemia times of up to 4 h [10].

Elevated concentrations of platelet activating factor (PAF), released by circulating polymorphonuclear leucocytes and pulmonary macrophages, account for pulmonary tissue injury resulting in bronchoconstriction, oxygen free radical generation, elevated pulmonary vascular resistance and microvascular leakage [11, 12]. Synthetic PAF-antagonists (PAF-a) were initially developed for the treatment of small airways disease [13], but due to the major involvement in the pathophysiology of pulmonary dysfunction various other indications – septic shock, graft rejection, pulmonary preservation and transplantation – were investigated throughout further evaluation [11, 13–15]. Since most of these problems are encountered in reperfusion injury we investigated the prevention of PAF-induced pulmonary dysfunction after lung transplantation in a canine model by the addition of a PAF-a (WEB 2170 BS). This was combined with our current clinical setup for lung preservation as represented by pulmonary artery flush with EC after previous systemic administration of PGI_2.

Materials and methods

Twenty-four mongrel dogs were divided in 2 groups of six donors and six weight-matched recipients each. All animals were cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

The dogs were anaesthetized and ventilated with 40% oxygen and 60% nitrous oxide using a positive endexspiratory pressure



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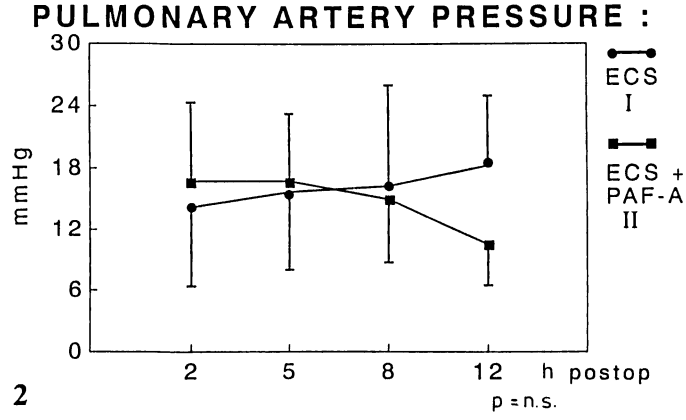

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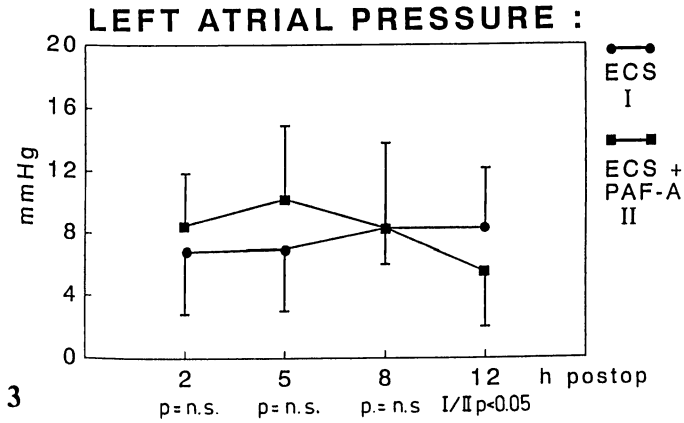

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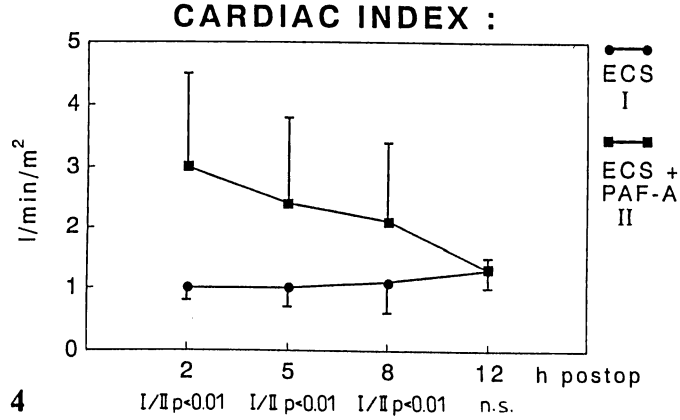

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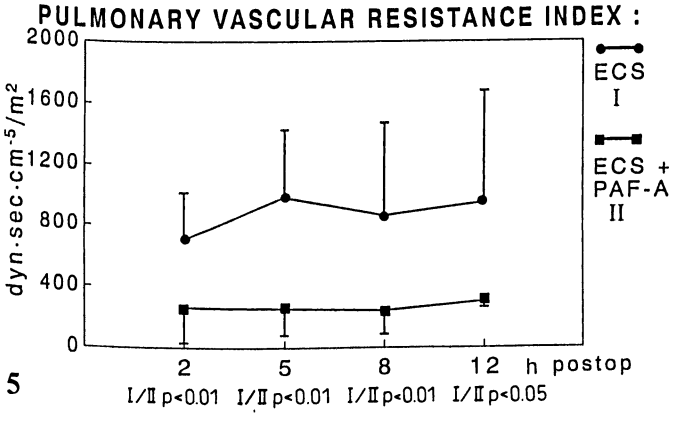

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Fig. 1. Postoperative donor left atrial pO_2-values (mean ± SD)

Fig. 2. Postoperative donor mean pulmonary artery pressure (PAP) (mean ± SD)

Fig. 3. Postoperative donor left atrial pressure (LAP) (mean ± SD)

Fig. 4. Postoperative donor cardiac index (CI) (mean ± SD)

Fig. 5. Postoperative donor pulmonary vascular resistance index (PVRI) (mean ± SD)

(PEEP) of 5 cm H_2O. The operative technique for donor and recipient operation has been described previously by Wahlers et al. [6]. Donor animals were divided into 2 groups according to the method of pulmonary preservation. In both groups pulmonary artery flush perfusion was performed with EC (60 ml/kg at 4 °C) after previous intravenous administration of PGI_2 over a 10 min period (20 ng/kg per min), while additionally, in group II, WEB 2170 BS was administered to the donor, added to the storage solution and administered to the recipient during reperfusion (Table 1). For cardioplegia, St. Thomas Hospital solution (20 ml/kg at 4 °C) was used in all donors. The organ block was immersed in cold EC without further dissection and stored at 4 °C for about 5 h.

The recipient animals were prepared during the organ storage period. Briefly, a left pneumonectomy was performed and the donor heart with the adjacent left lung were transplanted as has been described in earlier studies [6, 16]. Anastomoses were performed end-to-side between the donor's and recipient's superior vena cava (SVC) and the donor's descending aorta was sutured end-to-side to the proximal part of the recipient's descending aorta. Ligation of the recipient's SVC proximal to the anastomosis after 30 min of reperfusion provided a separation of the donor and recipient pulmonary circulation. The donor's trachea was intubated with an endotracheal tube inserted through the 2nd intercostal space and ventilated separately (oxygen 40 %/nitrous oxide 60 %, tidal volume: 10 cc/kg, respiratory rate: 12/min, PEEP: 5 cm H_2O). The chest was closed temporarily.

Arterial pressure (AP) was measured in the carotid artery, and heart rate (HR), central-venous pressure (CVP), pulmonary artery pressure (PAP), left atrial pressure (LAP), cardiac output (CO) and left atrial blood gas analysis (BGA) were obtained separately every hour in the donor's and the recipient's circulation until the postoperative course was terminated after 12 h or the animal died. Calcula-

tion of cardiac index (CI) and pulmonary vascular resistance index (PVRI) were performed according to standard formula. Data are expressed as mean values ± standard deviation (SD). To exclude influences of baseline data on postoperative results, analysis of covariance (ANCOVA) was performed. P values less than 0.05 were considered significant.

Results

No significant differences were found between the donor data – except for the oxygenation – and the ischemia time (Table 2). The CVP was adjusted in both groups to approximately 6–8 mm Hg for the entire postoperative course. No recipient animal died during the first 8 h postoperatively, however one animal in the EC group (group I) and two dogs in the EC/PAF-a group (group II) died between 8 and 12 h postoperatively. Causes for premature deaths were progressive cardiac failure or dysrhythmias in all cases.

Significantly improved oxygenation of the donor lung after transplantation was observed in the EC/PAF-a (group II) group compared to the EC group (group I) throughout the entire postoperative period (Fig. 1). In both groups mean values of left atrial pO_2 were stable during the first 6 h after transplantation and decreased significantly in the later postoperative course from 190 ± 56 mm Hg at 2 h to 123 ± 34 mm Hg at 12 h in group I ($P < 0.05$), and 296 ± 17 mm Hg at 2 h and 223 ± 47 mm Hg at 12 h in group II ($P < 0.05$). Comparing mean pO_2 values of the donor lung before harvesting and early postoperatively, a reduction in oxygenation of 61 % was noted in group I versus only 23 % in group II.

Mean postoperative PAPs ranged from 14.2 ± 7.3 mm Hg at 2 h to 18.3 ± 6.7 mm Hg at 12 h in group I, and from 16.8 ± 7.3 mm Hg at 2 h to 10.5 ± 4.9 mm Hg at 12 h in group II (Fig. 2). While in group I a slight, but not significant increase was found in PAP throughout the postoperative period, PAP decreased in group II, but this was not significant. Comparing the corresponding postoperative PAP values of both groups, no significant differences were found at any time. Analysis of postoperative LAP values of both groups revealed a statistically significant difference only at 12 h postoperatively (group I: 8.3 ± 3.9 mm Hg versus 5.5 ± 3.5 mm Hg in group II, $P < 0.05$) (Fig. 3).

Cardiac index (CI) was elevated in group II compared to group I and this difference was significant during the first 8 h postoperatively (CI at 2 h: group I, 1.06 ± 0.29 l/min/m^2, group II, 2.98 ± 1.45 l/min/m^2, $P < 0.01$; at 5 h: group I, 1.04 ± 0.43 l/min/m^2, group II, 2.41 ± 1.38 l/min/m^2, $P < 0.05$; and at 8 h: group I, 1.15 ± 0.62 l/min/m^2, group II, 2.12 ± 1.32 l/min/m^2, $P < 0.05$). While CI was stable in both groups during this time, in the later postoperative course CI decreased in group II ($P < 0.05$). Despite this decrease in CI, values in group II were still elevated compared with group I, but the differences were no longer significant (CI at 12 h: group I, 1.05 ± 0.23 l/min/m^2; group II, 1.32 ± 0.20 l/min/m^2, n.s.) (Fig. 4).

The pulmonary vascular resistance index (PVRI) was elevated postoperatively in both groups and ranged between 705 ± 313 dyn s cm^{-5} m^2 at 2 h and 947 ± 732 dyn s cm^{-5} m^2 at 12 h in group I and between 296 ± 268 dyn s cm^{-5} m^2 at 2 h and 300 ± 40 dyn s cm^{-5} m^2 at 12 h in group II (Fig. 5). PVRI values in group I were elevated at all times postoperatively compared with the corresponding values for group II. These differences reached statistical significance at 2, 5, and 8 h postoperatively.

Between the two groups no significant differences were found in the recipient data during the postoperative course comparing corresponding values of CI, PAP, LAP and PVRI, except for a significant decrease in the pO_2 values in both groups ($P < 0.05$).

Table 1. Groups investigated

I ($n = 6$)	Pulmonary artery flush with cold Euro-Collins-solution (60 ml/kg) after prostacyclin pretreatment (20 ng/kg/min) (storage: ECS)
II ($n = 6$)	Pulmonary artery flush (≙ I) + PAF-antagonist WEB 2017 BS (Donor: 0.15 mg/kg/30 min, recipient: 0.3 mg/kg/h for 6 h, Storage solution: ECS + 0.3 mg/kg WEB 2170 BS)

Table 2. Donor and procurement data (mean ± SD)

	Group I (EC)	Group II (EC/PAF-a)	
Body weight (kg)	23.2 ± 2.8	23.8 ± 3.8	n.s.
PAP (mm Hg)	15.7 ± 4.8	16.8 ± 5.0	n.s.
LAP (mm Hg)	9.0 ± 3.4	7.3 ± 4.6	n.s.
CI (l/min/m^2)	3.89 ± 1.07	3.45 ± 1.50	n.s.
PVRI (dyn s cm^{-5} m^2)	197 ± 29	196 ± 85	n.s.
pO_2 (mm Hg)	190 ± 10	270 ± 25	$P < 0.01$
Perfusion pressure (mm Hg)	13.5 ± 5.5	11.7 ± 3.4	n.s.
Ischemia time (min)	368 + 30	338 + 18	n.s.

PAP, pulmonary artery pressure; LAP, left atrial pressure; CI, cardiac index; PVRI, pulmonary vascular resistance index; pO_2, arterial oxygen tension; EC, Euro-Collins solution; PAF-a, PAF antagonist; n.s., not significant

Discussion

In the past, different methods and techniques for lung procurement and preservation have been developed and used in clinical transplantation. However, donor core cooling by means of extracorporeal circulation [5, 17] and single flush perfusion of the pulmonary artery using either Euro-Collins solution [2, 6–10] or cold blood [4], are currently used by most lung transplant centers. The EC flush is combined with the systemic administration of prostaglandins to the donor immediately before harvesting, since Jurmann et al. have demonstrated that the addition of PGI_2 provides pulmonary vasodilatation resulting in better distribution of the crystalloid flush and improved postoperative graft function [18]. Despite satisfactory clinical results in lung transplantation, with mean ischemia times of up to 4 h [10] multiple efforts have been made to prolong the ischemic tolerance of the lung. In particular, the

use of oxygen free radical scavengers [19, 20] and the use of University of Wisconsin solution for lung preservation [21] have been studied recently.

We investigated, in canine model, the effects of the PAF-antagonist WEB 2170 BS in combination with EC flush perfusion after PGI_2 pretreatment on the postoperative graft function compared to EC + PGI_2 alone. In both experimental groups, EC was administrated with a perfusion volume of 60 ml/kg at 4 °C and perfusion pressures according to the native PAP were used, since Haverich et al. have demonstrated the advantages of the high volume low pressure pulmonary artery flush with respect to homogeneous fluid distribution and uniform organ cooling [7]. Heterotopic heart- and orthotopic single lung-transplantation was performed, since extracorporeal circulation can be omitted throughout the implantation [6, 16]. Another advantage of the model is based on the separation of donor and recipient circulation, because organ deterioration does not directly lead to the recipient's death and reperfusion injury can be studied more extensively.

For quantification of lung preservation quality, left atrial pO_2, PAP and PVRI were considered, since various experimental studies have demonstrated that these parameters are most suitable for assessment of posttransplant lung function [6, 7, 22]. Analysis of lung water content was not performed, because a minor sensitivity can be expected when compared to functional parameters [23]. Our results demonstrated that the use of WEB 2170 BS in combination with EC flush resulted in a significantly improved postoperative oxygenation of the transplanted lung. In addition, comparing donor pO_2 values before harvesting and early postoperatively only the slightest impairment of oxygenation was noted in the EC/PAF-a group. Comparing corresponding PAP and LAP values between both groups and throughout the postoperative course no significant differences or changes were observed except for the PAP at 12 h postoperatively ($P < 0.05$). However, using identical myocardial protection in both groups, the higher CI in the EC/PAF-a group without corresponding increase in PAP probably reflects a better preservation of the pulmonary vascular bed resulting in a significantly lower PVRI during the first 8 h postoperatively. Similar results have been described by Conte and Foegh using the PAF-a BN 52021 in long term lung preservation in a canine model [14].

The excellent post-transplant lung function using the PAF-a WEB 2170 BS might be due to improved donor organ preservation as well as reduced reperfusion injury. It can be speculated that the PAF release by circulating leucocytes and alveolar macrophages is probably one of the most important pathophysiologic pathways activated during perfusion, ischemic storage and reperfusion of the lungs. PAF-induced oxygen free radical generation and liberation of vasoactive substances may be attenuated by the addition of PAF-antagonists resulting in better oxygenation and lower pulmonary vascular resistance.

In conclusion, the use of the PAF-a, WEB 2170 BS, in combination with EC flush in lung transplantation provided significantly improved postoperative oxygenation and lower pulmonary vascular resistance when administered to the donor before harvesting, to the storage solution and to the recipient during reperfusion as demonstrated in a canine heterotopic heart- and orthotopic left lung-transplant model after 6 h of cold ischemia.

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