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Treatment with a platelet-activating factor receptor antagonist improves hemodynamics and reduces epinephrine requirements, in a lethal rodent model of anaphylactic shock

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Abstract

Background: In some cases, anaphylactic shock (AS) is still lethal, despite rapid use of epinephrine. High doses of epinephrine are associated with severe complications. Platelet-activating factor (PAF) is secreted in massive amounts during AS, and a high plasma level is correlated with increased AS severity.

Objective: To assess the effect of ABT-491, a PAF-receptor antagonist and possible adjunct treatment, alone or in combination with epinephrine during AS.

Methods: AS was induced by intravenous injection of 1 mg ovalbumin into ovalbumin-sensitized rats. Rats were then randomly assigned to 5 groups (n = 10 per group): SHAM (vehicle only), SHOCK (no treatment), ABT (ABT-491 1 mg/kg), EPI (epinephrine 5 µg as a bolus then 10 µg kg⁻¹ min⁻¹ by continuous infusion, followed by a reducing protocol) and EPI-ABT (both treatments).

Results: Ovalbumin injection resulted in a severe decrease in mean arterial pressure, left ventricular inotropy (max dP/dt) and left ventricular shortening fraction (LVSF). All rats from the ABT group survived until the end of the experiment. ABT-491 prevented the LVSF decrease observed in the SHOCK group (at T15: ABT 50% ± 11% vs SHOCK 36% ± 9%, P = .01), significantly reduced the dose of epinephrine needed to treat anaphylactic shock (EPI-ABT 314 ± 67 µg/kg vs EPI 475 ± 69 µg/kg, P < .001) and reduced the time to restore basal MAP (ABT 23 ± 7 minutes vs EPI-ABT 13 ± 5 minutes, P < .01).

Conclusions and Clinical Relevance: AS was characterized by early cardiac dysfunction in our model. Treatment with ABT-491 allowed survival until the end of the experiment and reduced cardiac dysfunction. Use of the PAF-R antagonist had a synergistic effect with epinephrine and allowed a significant reduction in epinephrine consumption. Use of PAF-R antagonists during AS could reduce epinephrine-related complications and improve the treatment of epinephrine refractory cases.

KEYWORDS

anaphylaxis, epinephrine, ovalbumin, platelet-activating factor, shock

1 | INTRODUCTION

Anaphylactic shock (AS) is a severe systemic hypersensitivity reaction following exposure to an antigen. In Europe, the incidence of anaphylaxis is estimated at 1.5–32 for 100 000 person-years.¹ During anaesthesia, the incidence of perioperative anaphylaxis ranges from 1/1250 to 1/18 600 procedures.² Mortality rate of perioperative anaphylaxis ranges from 0% to 1.4% in Western Australia (2000–2009),³ to 4% in USA and Japan.^{4,5} In the US, 500 to 1000 deaths are attributed to anaphylaxis every year.⁶ Interestingly, Reitter et al reported a 4.1% mortality rate after neuromuscular blocking agent (NMBA) related anaphylaxis, despite fully monitored patients and rapid injection of epinephrine.⁷ Epinephrine is the recommended first-line treatment in cases of anaphylaxis. The level of evidence for its use is low, based mainly on expert opinion and experimental studies.⁸ Its use was extrapolated from other clinical situations where high doses are currently questioned.^{9,10} During AS, the injection of high doses of epinephrine; especially intravenously, is associated with severe complications such as arrhythmia, myocardial infarction and stress cardiomyopathy.^{11,12} Epinephrine should therefore be carefully titrated according to dosing recommendations for AS, to restore adequate circulation and by IM or IV administration as clinically appropriate.

The relatively high mortality rates indicate that some AS is refractory to epinephrine and new therapeutic strategies are needed. Combined therapy with an agent which has a synergistic effect with epinephrine appears to be a good way to reduce epinephrine dose and thereby unwanted side-effects.

Platelet-activating factor (PAF) is a potent phospholipid that plays a central role in immune and inflammatory responses. PAF is massively released during anaphylaxis by numerous cells including platelets, basophils and mast cells and contributes to hypotension due to its vasodilator and negative inotropic effects.¹³ In human anaphylaxis, serum PAF levels are correlated with the severity of the reaction whereas PAF-acetylhydrolase levels (PAF-AH), which partially controls circulating PAF activity by hydrolysing PAF into a biologically inactive form, are inversely correlated to severity.¹⁴ Blockade of the PAF-receptor (PAF-R) has not previously been tested during AS resuscitation.

The goal of our study was to assess the hemodynamic effects of ABT-491, an antagonist of PAF-R, alone or in combination with epinephrine, in a rodent model of lethal ovalbumin-induced anaphylactic shock.

2 | METHODS

All animal procedures and care were performed in accordance with the European Communities Council Directive of 2013 (Directive 2010/63/EU), the official recommendation of the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. This study was ethically reviewed and approved by the ethical committee of Strasbourg (File AL/105/112/02/13). Ten-week-old Brown Norway rats weighing 250–350 g (Charles River Laboratories France)

were used for this experiment. Animals were kept under standard conditions (temperature $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$; lights from 6:00 AM to 6:00 PM) and given a standard diet (A04; UAR) and water ad libitum. Rats were sensitized by subcutaneous administration of 1 mg intravenous (IV) chicken egg albumin, ovalbumin, (OVA; Sigma-Aldrich) and 4 mg aluminium hydroxide ($\text{Al}(\text{OH})_3$; Sigma) diluted in 1 mL 0.9% saline solution on days 0, 7 and 14, as described previously.¹⁵

The surgical procedure was performed on day 21 following the initial sensitization. Anaesthesia was induced with 4% isoflurane and maintained with 1% isoflurane. Rats were tracheostomized and mechanically ventilated with 100% oxygen using a rodent respirator (Alpha Lab, Equipement Vétérinaire Minerve). Tidal volume and respiratory rate was adjusted to maintain PaCO_2 between 5.1 and 5.6 kPa at the beginning of the experiment. Rectal temperature was maintained at $38.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ by a heating pad during the whole experiment. A fluid-filled polyurethane catheter was inserted into the right jugular vein for ovalbumin injection and drug administration. A continuous infusion of 0.9% saline was started at T0 at $30 \text{ mL kg}^{-1} \text{ h}^{-1}$ in all shocked groups.

A fluid-filled polyurethane catheter was inserted into the left carotid artery for pressure monitoring. Systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) were recorded continuously using a strain gauge pressure transducer linked to Powerlab (ADInstrument). A polyimide Mikro-Tip pressure catheter (SPR-320NR, Millar Inc) was inserted into the right carotid artery and moved into the left ventricle for continuous pressure monitoring. Max dP/dt , which reflects left ventricle inotropy, was calculated with the LabChart 7 software (ADInstrument).

Left ventricular function was assessed at T0 (just before ovalbumin injection), then at T2.5 (2.5 minutes after ovalbumin injection), T5, T10, T15, T20 and then every 10 minutes after ovalbumin injection with cardiac echography (Vivid 7, GE healthcare France) using a 12S probe. Left ventricular end-diastolic diameters (LVEDD) were assessed in the short-axis view. The image was considered valid if the left ventricle appeared circular with a visualization of papillary muscle. Left ventricular shortening fraction (LVSF) was calculated as follows: $(\text{LVEDD} - \text{LVESD}) / \text{LVEDD} \times 100$.

A flexible Clark-type polarographic oxygen (pO_2 Microprobe 200 mm, Integra LifeScience) connected to a Licox system (Integra LifeScience) was inserted into the left gastrocnemius muscle for PtiO_2 measurement.

After a fifteen-minute stabilization period following the end of the surgical procedure, anaphylactic shock was induced by intravenous injection of 1 mg ovalbumin. Animals were randomly assigned to five different groups:

- SHAM (non-shocked group): vehicle only.
- SHOCK: no treatment, saline infusion only.
- ABT: injection of 1 mg/kg ABT-491 (Sigma-Aldrich) diluted in 1 mL 0.9% saline at T3.
- EPI: injection of 2 boluses of epinephrine (Adrenaline Aguettant) $2.5 \mu\text{g}$ diluted in 0.5 mL 0.9% saline at T3 and T5 followed by a continuous infusion of epinephrine started at $10 \mu\text{g kg}^{-1} \text{ min}^{-1}$ as described previously.¹⁶ Epinephrine continuous infusion was then

reduced by $0.5 \mu\text{g kg}^{-1} \text{min}^{-1}$ each five minutes if MAP was above 100 mm Hg for at least 5 minutes.

- EPI-ABT: combined treatment group, ABT-491 was injected with the first bolus of epinephrine. Epinephrine treatment protocol was the same as in the EPI group.

The dose of 1 mg/kg of ABT-491 was based on previously published pharmacological studies.¹⁷

MAP, dP/dt and $PtiO_2$ were recorded at the following time-points: T0 (before ovalbumin injection), T1, T2.5, T5, T7.5, T10, T15, T20 and then every 5 minutes until T60.

Arterial blood samples were taken from each animal before shock induction and at the end of the experiment for measurement of arterial blood gas and haematocrit.

At the end of the experiment, after the last blood sampling, rats were killed by an anaesthetic overdose. As described previously, without treatment, AS is lethal within 20 minutes after shock induction.¹⁸ In the SHOCK group, rats were all killed at T15. Rats in the remaining groups were killed at T60.

The PAF-acetylhydrolase (PAF-AH) hydrolyzes and inactivates PAF. The PAF-AH activity is inversely correlated with plasma PAF levels in humans.¹⁴ In order to confirm the secretion of PAF in our model, we measured extracellular PAF-AH activity in SHAM and SHOCK groups using a commercial chromogenic kinetic kit (Abcam). Briefly, hydrolysis of a modified substrate (2-thio-PAF) by the plasma PAF-AH released a coloured product after conjugation with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). Optical absorbance was measured at 414 nm once every 30 seconds to get 10 time-points. Results were calculated using the following formula: $[\text{PAF-AH} (\mu\text{mol min}^{-1} \text{mL}^{-1}) = \Delta A_{414} \text{min}^{-1} \times 0.225 \text{ mL} / 10.66 \text{ nM} / 0.01 \text{ mL} \times \text{sample dilution}]$. Results were expressed in $\text{nmol min}^{-1} \text{mL}^{-1}$.

3 | STATISTICAL ANALYSIS

Variables were expressed as mean $\pm$ standard deviation then compared between all groups using ANOVA. Gaussian distributions were assessed graphically and by using Shapiro-Wilk's test.

Post hoc analyses were performed for the significant variables in ANOVA to determine which groups differed from each other, using Tukey's Honest Significant Difference test.

Linear mixed models enabled us to evaluate the evolution of variables over time for each group, by fitting a polynomial of degree 2 for the time.

The significance level was set at 5%.

Statistical analyses were performed using the R software version 3.4.0.

4 | RESULTS

Fifty 10-week-old brown Norway rats were randomly allocated into the five groups ($n = 10$ per group).

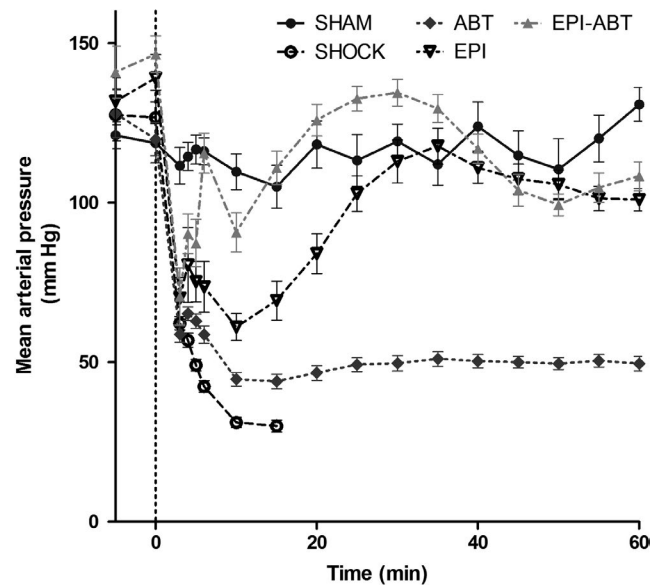


FIGURE 1 Time course of mean arterial pressure in SHAM (vehicle only, no shock), SHOCK (shock, no treatment), ABT (treatment with ABT-491), EPI (treatment with epinephrine) and EPI-ABT (treatment with both epinephrine and ABT-491) groups ($n = 10$ per groups). Ovalbumin was injected at T0. A sharp decrease was observed in all shocked groups while MAP remained stable during the whole experiment in the SHAM group. All rats treated with ABT-491 (ABT) survived until the end of the experiment. Treatment with epinephrine and ABT-491 (EPI-ABT) resulted in a significant improvement in MAP when compared with treatment with epinephrine only (EPI) ($P = .01$ at T15 and T30). Results are represented as mean $\pm$ SEM

4.1 | PAF-AH activity

The level of PAF-AH activity was significantly lower in the SHOCK group when compared with the SHAM group (respectively 8.7 ± 2.6 vs $10.3 \pm 1.5 \text{ nmol min}^{-1} \text{mL}^{-1}$; $P = .001$) indicating that PAF is secreted during ovalbumin-induced anaphylaxis in our model.

4.2 | Hemodynamic parameters

All parameters remained constant during the whole experimentation period in the SHAM group. In the SHOCK, ABT, EPI and EPI-ABT groups, ovalbumin injection resulted in a similar severe decrease in MAP and dP/dt at T3 before treatment injection ($p = \text{ns}$) (Figures 1 and 2).

After ovalbumin injection, MAP reached a minimal value of $30 \pm 6 \text{ mm Hg}$ in the SHOCK group at T15 and stabilized around $44 \pm 15 \text{ mm Hg}$ in the ABT group. In this group, all rats survived until the end of the experiment. In the EPI and EPI-ABT groups, after a transient increase in MAP due to epinephrine boluses, MAP progressively increased to reach a maximum value of $118 \pm 18 \text{ mm Hg}$ in the EPI group and $136 \pm 14 \text{ mm Hg}$ in the EPI-ABT group ($P < .05$). At the end of the experiment, MAP remained low in the ABT group, with a

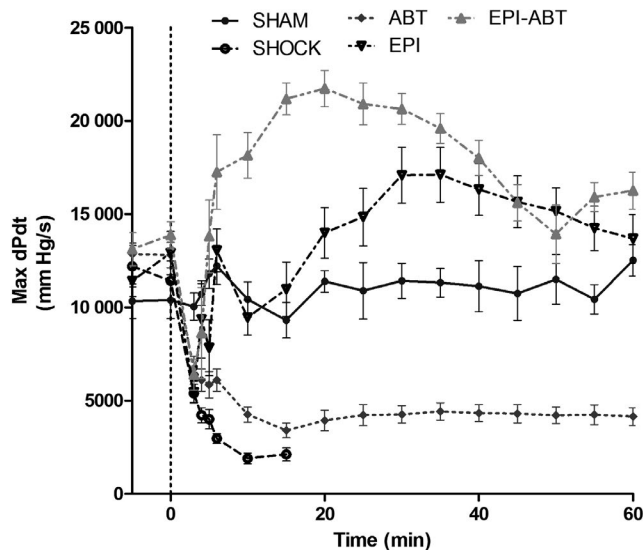


FIGURE 2 Time course of max dP/dt in SHAM (vehicle only, no shock), SHOCK (shock, no treatment), ABT (treatment with ABT-491), EPI (treatment with epinephrine) and EPI-ABT (treatment with both epinephrine and ABT-491) groups ($n = 10$ per groups). Ovalbumin was injected at T0. A sharp decrease was observed in all shocked groups while max dP/dt remained stable during the whole experiment in the SHAM group. At T15, max dP/dt was not statistically different between SHOCK and ABT groups ($P = .8$). The combination of epinephrine and ABT-491 (EPI-ABT) resulted in a significantly higher max dP/dt at T15 when compared with epinephrine alone (EPI) ($P < .01$). Results are represented as mean $\pm$ SEM

value of 50 ± 17 mm Hg. Time to reach 100 mm Hg was significantly shorter with EPI-ABT vs EPI alone (13 ± 5 minutes vs 23 ± 7 minutes, $P < .001$). Total epinephrine consumption was significantly lower with the EPI-ABT group than with EPI alone (314 ± 67 μ g/kg vs 475 ± 69 μ g/kg, $P < .001$).

Baseline max dP/dt was similar between groups before ovalbumin injection (Figure 2). Max dP/dt remained stable during experimentation in the SHAM group with a value of $12\,527 \pm 5684$ mm Hg/s at the end of the experimentation. Max dP/dt reached 2120 ± 1260 mm Hg/s at T15 ($P < .001$ vs SHAM) in the SHOCK group. In the ABT group, max dP/dt reached its minimum at T15 and then stabilized at 4150 ± 1520 mm Hg/s until the end of the experiment. In the EPI and EPI-ABT groups, max dP/dt reached their minimum before treatment injection at T3 (respectively 5460 ± 2380 and 6400 ± 3560 mm Hg/s; $p = \text{ns}$). In the EPI group, after a transient effect of epinephrine boluses, max dP/dt progressively increased to reach a maximum at T30 ($17\,270 \pm 6760$ mm Hg/s). In the EPI-ABT group, the maximum value of dP/dt was reached at T20 ($21\,730 \pm 10\,710$ mm Hg/s). Time to reach 10 000 mm Hg/s was significantly shorter in the EPI-ABT group than in the EPI group (5 ± 1 minute vs 14 ± 6 minutes; $P < .01$). At T60 minutes, no significant differences were observed between the SHAM, EPI and EPI-ABT groups. In the ABT group, max dP/dt remained significantly lower in comparison with the SHAM group ($P < .001$).

4.3 | Echocardiography parameters

In all shocked groups, left ventricular end-diastolic diameters (LVEDD) severely decreased just after shock induction to reach a minimum value of 3.9 ± 0.7 mm in the SHOCK group, 4.2 ± 0.8 mm in the ABT group, 4.0 ± 0.7 mm in the EPI group and 4.3 ± 0.5 mm in the EPI-ABT group (Figure 3A). In all treated groups, LVEDD was not statistically different at any time during the experiment. In the SHOCK group, left ventricular shortening fractions (LVSF) showed a marked decrease at T10 and was at $36\% \pm 9\%$ at T15 (vs SHAM $48\% \pm 5\%$; $P < .05$) (Figure 3B). In the ABT group, LVSF remained similar to the SHAM group during the whole experiment. In epinephrine treated groups (EPI and EPI-ABT), LVSF increased after epinephrine injection and remained significantly higher than for the SHAM group throughout the whole experiment ($P < .01$ for both groups). There were no significant differences between the EPI and EPI-ABT groups.

4.4 | Metabolic parameters

Muscular PtiO₂ remained stable during the whole experiment in the SHAM group (Figure 4). In the SHOCK, ABT, EPI and EPI-ABT groups a marked decrease in PtiO₂ was observed after shock induction. The minimal level of PtiO₂ was 12 ± 12 mm Hg in the SHOCK group, 14 ± 12 mm Hg in the ABT group, 11 ± 10 mm Hg in the EPI group and 19 ± 14 mm Hg in the EPI-ABT group. In treated groups, PtiO₂ progressively increased to reach 27 ± 18 mm Hg in the ABT group, 14 ± 14 mm Hg in the EPI group and 27 ± 14 mm Hg in the EPI-ABT group at T60 without significant difference. Interestingly, muscular PtiO₂ showed a tendency to improve in groups treated with ABT-491 (ABT and EPI-ABT) at the end of the experiment.

No significant differences were observed before shock induction in blood gases parameters (Table 1). In the absence of treatment, shock was characterized by a sharp increase in blood lactate level (7.5 ± 1.4 mmol/L) at T15. Compared with the SHOCK group at T15, the lactate increase at T60 was lower in the ABT, EPI and EPI-ABT groups, with respectively 4.4 ± 1.2 , 5.0 ± 1.9 and 3.7 ± 0.9 mmol/L ($P < .001$). Lactate was not statistically different between treated groups at T60.

5 | DISCUSSION

AS is classically described as a distributive shock, with a sharp decrease in peripheral vascular resistance and severe microvascular leakage, leading to profound hypovolaemia. These elements are responsible for a severe decrease in MAP and cardiac output (CO). Exploring beyond this description of AS, our study brings new insights into the pathophysiology of AS and revealed that early cardiac dysfunction is present during AS, shown by the severe drop in max dP/dt and LVSF in the SHOCK group.

Low end-tidal CO₂ (EtCO₂) has recently been proposed as a severity marker to identify severe AS during anaesthesia.¹⁹ Our study

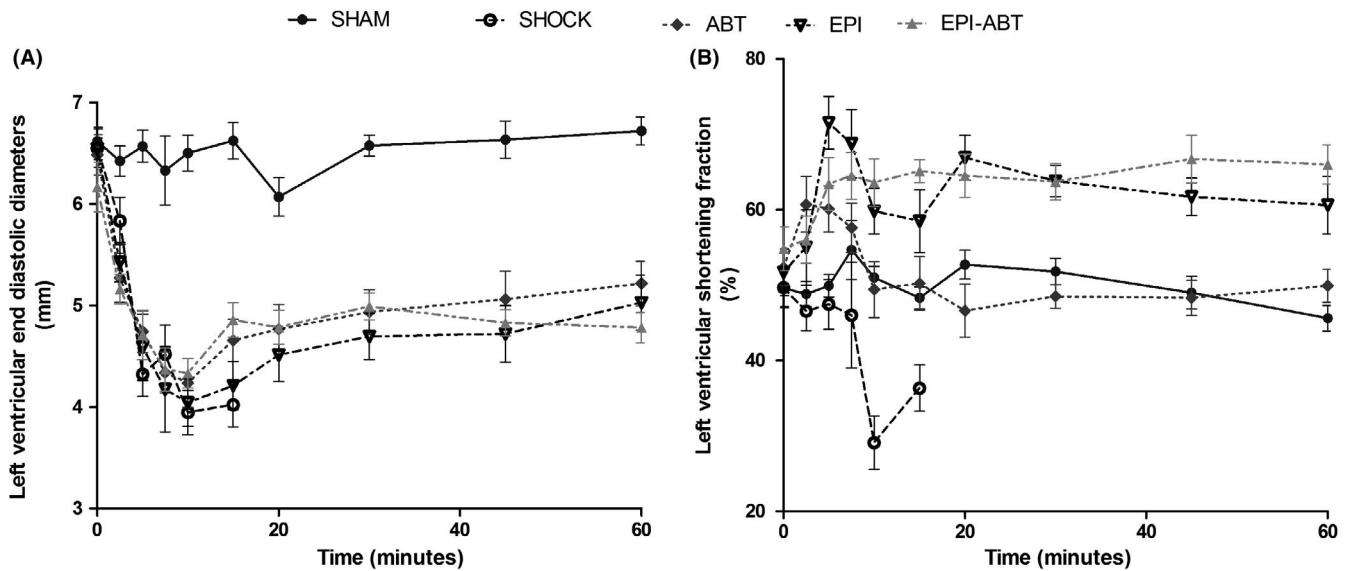


FIGURE 3 Time course of echocardiography parameters in SHAM (vehicle only, no shock), SHOCK (shock, no treatment), ABT (treatment with ABT-491), EPI (treatment with epinephrine) and EPI-ABT (treatment with both epinephrine and ABT-491) groups ($n = 10$ per groups). A, left ventricular end-diastolic diameters (LVEDD). While LVEDD remained stable during the whole experiment in the SHAM group, ovalbumin injection (T0) resulted in a significant decrease in LVEDD in all shocked groups (SHOCK, ABT, EPI, EPI-ABT) ($P < .01$ in comparison with the SHAM group). Despite subsequent vascular filling, LVEDD remained lower in all treated groups when compared with the SHAM group ($P < .01$ at T60). Results are represented as mean $\pm$ SEM. B, left ventricular shortening fraction (LVSF). Ovalbumin injection resulted in a decrease in the LVSF after 10 minutes in the untreated group (SHOCK) ($P < .05$ in comparison with SHAM). Treatment with ABT-491 alone (ABT) prevented the alteration of the LVSF ($P = .01$ at T15 in comparison with SHOCK). Treatment with epinephrine (EPI and EPI-ABT) resulted in a significant higher LVSF during the whole experiment ($P < .01$ at T60 in comparison with SHAM). The combination of epinephrine and ABT-491 was not statistically different than with epinephrine alone. Results are represented as mean $\pm$ SEM

suggests that the low EtCO_2 observed during AS is not only due to a low cardiac output, but also due to a lack of exchange between the adaptive compartments, especially the muscles and the vascular compartment. PaCO_2 was reduced in the SHOCK group while ventilation parameters remained constant. This low PaCO_2 could result from a reduction in extraction of CO_2 from the muscular compartment. While PaO_2 remained high, we observed a severe decrease in muscular PtO_2 indicating that (a) exchange between the two compartments was impaired and (b) energy consumption was aberrantly persistent in non-working muscle. In a previous study using microdialysis, our group has shown that this persistent energy consumption led to a complete failure of cellular energy production, with an increased lactate/pyruvate ratio which could contribute to rapid organ dysfunction.¹⁵ Our results confirm the need to monitor EtCO_2 during AS in intubated patients, which reflects simultaneously the alteration of cardiac output and the perfusion of adaptive compartments.

Epinephrine is currently the recommended treatment for AS. As expected, treatment with epinephrine improved MAP, max dP/dt and LVSF indicating that epinephrine remains the first-line treatment for anaphylaxis (Figures 1, 2 and 3B). However, high doses of epinephrine were necessary to reach our hemodynamic goals in the EPI group, exposing animals to epinephrine-related complications. Moreover, epinephrine was unable to improve the perfusion of the muscular compartment (Figure 4).

Many mediators have been implicated in anaphylaxis. Histamine was one of the first identified mediators in anaphylaxis, but the

treatment of anaphylaxis with histamine receptor antagonists was disappointing and did not result in improved survival.²⁰ Conflicting results have also been observed with blockers of the NO pathway, which is involved in anaphylaxis pathophysiology.^{20,21} While PAF has long been recognized as a mediator of anaphylaxis, interest in this molecule as a therapeutic target has increased with the publication of studies showing a correlation between the plasma level of PAF and the severity of the reaction.²² Several studies have successfully tried PAF-R antagonist as pretreatment in experimental models of anaphylaxis.^{23,24} For the first time, we have tested experimentally ABT-491, a selective PAF-R antagonist,²⁵ as a treatment of AS, alone or in combination with epinephrine. The effect of ABT-491 on the PAF-R has been well studied, and ABT-491 has a good specificity for the PAF-R.¹⁷ A recent structural study showed that ABT-491 stabilizes the PAF-receptor in an inactive state by locking helices II and IV in an inward conformation, thus decreasing the receptor's ability to signal via G protein.²⁶ PAF is an unstable lipid mediator, which is therefore difficult to measure. However, the decrease in PAF-AH activity in the SHOCK group confirmed that, in our model, PAF is secreted during anaphylaxis. Although our study was not designed to assess mortality, all shocked rats in the ABT group survived until 60 minutes, whereas rats classically die within 20 minutes after shock induction if left untreated.¹⁶

Only a few data are available on cardiac dysfunction during AS and most of them are case reports of coronary spasm²⁷ or stress

cardiomyopathy.²⁸ Among other mediators released by myocardial mast cells during AS, PAF is known to have a direct negative inotropic effect and could play an important role in this cardiac dysfunction.^{29,30} Ovalbumin injection resulted in a decrease in the LVSF after 10 minutes in the untreated group (SHOCK) ($P < .05$ in comparison with SHAM). Treatment with ABT-491 alone (ABT) prevented the initial alteration of the LVSF ($P = .01$ at T15 in comparison with SHOCK). This may have been due to a specific action on cardiomyocytes by preventing cardiac dysfunction. Throughout the experiment, LVSF values in the ABT group were not statistically different from those in the SHAM

group which may seem to contradict values of max dP/dt. However, LVSF is strongly influenced by cardiac after-load, which is greatly reduced during anaphylaxis. In the SHOCK group, LVSF only decreased 10 minutes after shock induction, while the maximum dP/dt decreased very rapidly after shock induction. This could be due to the after-load decrease that will prevent the earlier reduction in the LVSF. LVSF was only decreased in the SHOCK group at an advanced stage of cardiac dysfunction when the negative inotropic effect observed in anaphylaxis exceeds the decrease in after-load. By its action on PAF-receptor, ABT-491 could prevent this last step, thus avoiding a decrease in LVSF.

The haemodynamic effects of ABT-491, especially on MAP, were insufficient to consider its use without epinephrine. Treatment with epinephrine (EPI and EPI-ABT) resulted in a significantly higher LVSF during the whole experiment ($P < .01$ at T60 in comparison with SHAM). The combination of epinephrine and ABT-491 allowed a marked decrease in epinephrine requirements and a significant reduction in the time to reach baseline MAP. ABT-491 also enhanced the effect of epinephrine on cardiac function with a significant and early improvement in maximal dP/dt. The use of combined therapy led to a reduction in the duration of hypotension, which in turn could reduce neurological morbidity during AS, where the cerebral autoregulation is impaired. Davidson et al showed that cerebral blood flow decreased linearly with a decrease in MAP, making the time to restore an adequate MAP critical to avoid cerebral lesions.¹⁸

The use of lower doses of epinephrine during AS, could also reduce the risk of epinephrine-related adverse effects that might lead to significant morbidity, especially when the injected dose is high. During cardiac arrest, high doses of epinephrine, as currently recommended, may actually be harmful and ineffective.^{31,32} During anaphylaxis, high doses of intravenously injected epinephrine have been reported to cause cardiovascular complications including arrhythmia, myocardial infarction and stress cardiomyopathy.^{11,12} Several authors are reporting cases of stress cardiomyopathy following anaphylaxis, which could be attributed to the anaphylaxis itself, or to inappropriately high doses of epinephrine.^{28,33} Epinephrine remains the first-line treatment of

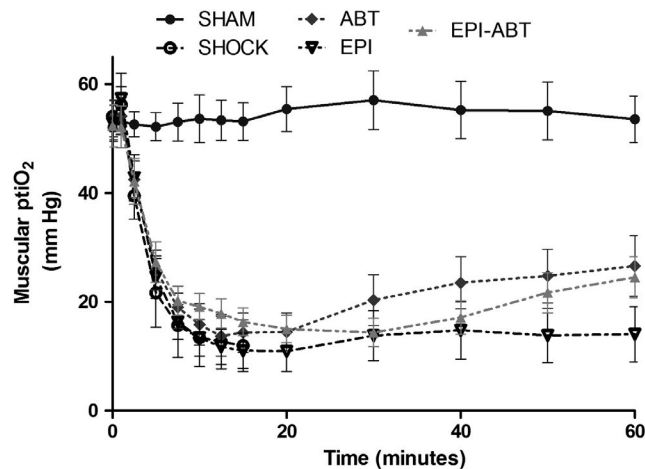


FIGURE 4 Time course of the right gastrocnemius muscular $PtIO_2$ in SHAM (vehicle only, no shock), SHOCK (shock, no treatment), ABT (treatment with ABT-491), EPI (treatment with epinephrine) and EPI-ABT (treatment with both epinephrine and ABT-491) groups ($n = 10$ per groups). Ovalbumin injection (T0) resulted in a sharp decrease in muscular $PtIO_2$ in all shocked groups (SHOCK, ABT, EPI, EPI-ABT). Neither ABT-491, epinephrine or a combination of the two treatments was successful in restoring muscular $PtIO_2$. Muscular $PtIO_2$ in groups treated with ABT-491 (ABT and EPI-ABT) showed a tendency towards improvement at the end of the experimentation period. Results are represented as mean $\pm$ SEM

Groups	Time	pH	PaO ₂ kPa	PaCO ₂ kPa	HT %	Lactate mmol/L
SHAM	T0	7.37 $\pm$ 0.04	28.8 $\pm$ 8.8	5.3 $\pm$ 1.8	39 $\pm$ 4	2.1 $\pm$ 1.0
	T60	7.37 $\pm$ 0.06	35.7 $\pm$ 13.1	5.5 $\pm$ 1.8	40 $\pm$ 3	1.5 $\pm$ 0.7
SHOCK	T0	7.39 $\pm$ 0.03	27.1 $\pm$ 10.4	5.1 $\pm$ 0.7	39 $\pm$ 3	1.9 $\pm$ 0.8
	T15	7.27 $\pm$ 0.03	34.8 $\pm$ 17.7	3.0 $\pm$ 1.5*	54 $\pm$ 6	7.5 $\pm$ 1.4
ABT	T0	7.37 $\pm$ 0.02	19.7 $\pm$ 6.9	5.4 $\pm$ 2.9	40 $\pm$ 2	1.7 $\pm$ 0.6
	T60	7.28 $\pm$ 0.04	15.3 $\pm$ 2.8	5.3 $\pm$ 2.8	42 $\pm$ 3	4.4 $\pm$ 1.2
EPI	T0	7.33 $\pm$ 0.03	23.1 $\pm$ 8.0	5.7 $\pm$ 3.2	38 $\pm$ 3	2.0 $\pm$ 0.9
	T60	7.12 $\pm$ 0.10	20.8 $\pm$ 9.2	7.3 $\pm$ 1.7*	46 $\pm$ 6	5.0 $\pm$ 1.9
EPI-ABT	T0	7.36 $\pm$ 0.02	17.2 $\pm$ 2.9	5.2 $\pm$ 2.7	37 $\pm$ 3	2.0 $\pm$ 0.8
	T60	7.16 $\pm$ 0.06	15.2 $\pm$ 4.4	7.6 $\pm$ 4.0*	43 $\pm$ 2	3.7 $\pm$ 0.9

Note: Results are expressed as mean $\pm$ SD. T0 corresponds to ovalbumin injection. HT, haematocrit.

* $P < .05$ vs SHAM at T60.

TABLE 1 Blood gas parameters of SHAM (vehicle only, no shock), SHOCK (shock, no treatment), ABT (treatment with ABT-491), EPI (treatment with epinephrine) and EPI-ABT (treatment with both epinephrine and ABT-491) groups ($n = 10$ per group)

anaphylactic shock, but the dose should be strictly adjusted according to severity and titrated to clinical response.

Our results show that, neither ABT-491, epinephrine, nor a combination of the two, were able to restore basal muscular $PtiO_2$. This incomplete reperfusion of the muscular compartment could be due to persistent and profound hypovolaemia, as shown by the persistently low LVEDD on echocardiography, despite vascular filling with 30 mL/kg of crystalloids, over 1 hour, in all treatment groups. PAF is known to increase vascular permeability,¹³ so we hypothesized that microvascular leakage could be reduced by PAF-R antagonists thus improving cardiac preload and muscle perfusion. The moderate effect observed of PAF-R blockade on $PtiO_2$ and LVEDD could be due to the delay between shock occurrence and ABT-491 administration or due to the impact of mediators other than PAF on vascular permeability.

As with any animal studies, any extrapolation to humans should be made carefully. However, clinical studies on human anaphylaxis are particularly difficult to design due to the rare and unpredictable occurrence of this complication, combined with the ethical issues of introducing a new treatment. Animal models therefore still have an important place, when testing new therapies for AS. PAF-R antagonists have already been tested in humans for indications other than anaphylaxis and showed a good safety profile, apart from in one study, which showed an increased risk of intra-cranial bleeding after subarachnoid haemorrhage.³⁴ Our study was not designed to assess the safety profile of ABT-491 but we did not observe significant bleeding during experimentation.

In conclusion, our study further explores new aspects of the pathophysiology of anaphylaxis. The existence of early cardiac dysfunction was confirmed. The perfusion of muscular compartments was severely impaired and resulted in a reduction in CO_2 extraction that may be contributing to the low $EtCO_2$ observed in human anaphylaxis.

Blockade of PAF, using a PAF-R antagonist appeared to be beneficial during AS. The effect of PAF-R antagonism was synergistic with epinephrine, reducing the duration of hypotension and the epinephrine dose needed to treat anaphylaxis.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

CT and WO made substantial contributions to the design of the work, the acquisition and the interpretation of data for the work. They drafted the work and gave their final approval of the version

submitted for publication. They were accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. OC, LHG, SN, BG and PMM made substantial contributions to the conception of the work and the interpretation of data for the work. They revisited it critically for important intellectual content and gave their final approval of the version submitted for publication. They were accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. NT made substantial contributions to the design of the work and the analysis of data for the work. He drafted the work and gave his final approval of the version submitted for publication. He was accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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