Platelet-activating factor (PAF)-induced cardiopulmonary dysfunctions and their reversal with a PAF antagonist (BN 52021) in strain 13 guinea pigs

Changgeng Qian, Zhong-Mao Guo, Clarence J. Peters and Ching-Tong Liu

Department of Clinical and Experimental Physiology, Disease Assessment Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA

(Received 18 August 1992)
(Revision received 10 November 1992)
(Accepted 23 November 1992)

Summary

Cardiovascular and respiratory responses to a 2 h intravenous constant infusion of PAF (5 and 10 ng/kg per min) were studied in strain 13 guinea pigs. PAF decreased arterial blood pressure, left systolic ventricular pressure, and cardiac output (CO). These cardiovascular changes were dose-dependent. The PAF-induced hypotension returned to a pre-infusion level spontaneously with increased total peripheral resistance despite continuous infusion of PAF. The decreased CO was most striking, and did not recover to pre-infusion levels due to depressed cardiac contractility and impaired ventricular relaxation. Respiratory responses to PAF infusion at these doses were mild and only occurred after serious cardiovascular dysfunctions developed. A higher dose of PAF (20 ng/kg per min) produced drastically decreased CO and dynamic lung compliance (Cml), increased pulmonary airway resistance, hypoventilation and apnea within 10–40 min. BN 52021, a PAF receptor antagonist, administered as a single i.v. dose (6 mg/kg) 15 min after PAF infusion, reversed most of cardiopulmonary dysfunctions and prevented death by increasing cardiac contractility, CO, and minute volume from extremely low values. The data suggest that marked cardiopulmonary disturbances induced
Best Available Copy
by intravenous PAF infusion reflects certain pathophysiological mechanisms of diseases that may involve the cellular release of PAF. The administration of BN 52021 or other potent PAF antagonists may be beneficial under these circumstances.

Key words: PAF; PAF antagonist; Cardiopulmonary function

1. Introduction

Platelet-activating factor (PAF) has been recognized as a potent mediator of endotoxin shock, cardiac anaphylaxis, asthma, inflammation and multi-organ dysfunctions (Koltai et al., 1991; Camussi et al., 1990; Snyder, 1990). Certain serious cardiovascular and respiratory dysfunctions have been demonstrated in isolated organs and after a single or short duration of intravenous administration of PAF at a very low dose level (ng/kg body weight) in experimental animals (Feuerstein, 1989; Barnes et al., 1989).

Strain 13 guinea pigs, inoculated with Pichinde virus, an arenavirus, have been used as an experimental model to mimic human Lassa fever (Jahrling et al., 1981; Peters et al., 1987). Reasons for selecting inbred (strain 13), instead of using outbred (Hartley), guinea pigs for the animal model have been previously reported (Liu, 1988a). Although strain 2 guinea pigs are also suitable for studying pathogenicity of arenaviral infection, most of our work and data were obtained from the strain 13 animal model. Particularly, unique cardiopulmonary dysfunctions were demonstrated during Pichinde viral infection in strain 13 guinea pigs (Peters et al., 1987; Liu, 1987). Also, PAF concentrations increased in the heart, lung, brain, and blood of Pichinde virus-infected strain 13 guinea pigs. These established results suggest that the cellular-released endogenous PAF may be involved in the development of cardiopulmonary disturbances during arenavirus-induced hemorrhagic fever (Qian et al., 1991; Liu et al., 1992).

In the present study, we evaluated cardiovascular and respiratory changes induced by a constant intravenous infusion of PAF at different doses in anesthetized strain 13 guinea pigs. Furthermore, we assessed the in vivo effectiveness of reversing PAF-induced cardiopulmonary changes by using BN 52021, a PAF receptor antagonist.

2. Materials and Methods

2.1. Animals

Male strain 13 guinea pigs, weighing 500–600 g, were obtained from the National Cancer Institute, Fort Detrick, MD. Guinea pigs were housed in a climate-controlled animal room (23°C) and provided with guinea pig chow (NIH-34M) and water ad libitum. Animals were anesthetized with Na pentobarbital (40
mg/kg, i.p.) before surgery, and the surgical level of anesthesia was maintained during the experiments by giving additional injections of Na pentobarbital (3.5 mg in 0.5 ml of saline solution, i.p.) when necessary. Body temperatures were maintained by a Harvard homeothermic blanket control unit (Harvard Apparatus, Edenbridge, KE) at 37.0°C during experiments.

2.2. Cardiovascular functions

A Y-shaped polyethylene catheter (PE-60, i.d. 0.58 mm) was inserted into the superior vena cava via the left external jugular vein. Two ends of the catheter were used to constantly infuse PAF and to inject 0.9% saline or BN 52021, separately. A Millar Micropipet SPR-40° (F-2) catheter pressure transducer (Millar Instruments, Houston, TX) was inserted into the left cardiac ventricle through the left common carotid artery. Heart rate (HR), left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were measured continuously for 2 h by a Buxco hemodynamic analyzer (Buxco Electronics, Sharon, CT). The maximum rate of changes of left ventricular pressure, including contraction and relaxation (+dP/dtmax and −dP/dtmax), were derived from the input signal of left ventricular pressure by the hemodynamic analyzer. These data were captured by a PC computer equipped with a Buxco Model 12 data logger.

The left common iliac artery was isolated by a retroperitoneal approach. A polyethylene catheter (PE-60, i.d. 0.86 mm) with a centric micro thermoprobe (Model RF-1, size 0.5 mm; Columbus Instruments, Columbus, OH) was made and introduced into the left common iliac artery. The catheter with the thermoprobe in the lumen sealed with glue was advanced into the aortic arch to measure arterial blood pressure (BP, systolic, diastolic and mean) and cardiac output (CO), which was measured by thermodilution. 200 μl of 0.9% NaCl solution at room temperature was automatically injected into the superior vena cava by a Micro Injector at 5-min intervals. A Cardiomax II R computer system (Columbus Instruments, Columbus, OH) computed CO values from the microprobe-sensed thermodilution curve. Cardiac output values were divided by the body surface area of each guinea pig (Liu, 1988b) to obtain the cardiac index (CI). Body surface area (cm²) = body weight (g) 0.25 x K. The K values varied from 11.318 to 9.060 according to the animal body weight which ranged from 80 to 880 g (Liu, 1988b).

2.3. Pulmonary functions

Respiratory functions were monitored and analyzed according to a modified method described by Brunet et al. (1983). Briefly, polyethylene tubing (i.d. 0.17 cm) was inserted into the trachea and connected to a Fleisch 3.0 pneumotachograph (whittaker, Blue Bell, PA), which was coupled to a Validyne DP54-14 differential pressure transducer (Validyne, Northridge, CA). After a small opening was made on the skin between the fifth and sixth intercostal space near the posterior mid-line, and after a small piece of intercostal muscle was removed, a pointed piece of PE 190 tubing (o.d. 1.7 mm) was pushed into the intrapleural space. Tissue adhesive (3M, St. Paul, MN) was placed around the tubing at the entrance site to secure and prevent air leakage. Transpulmonary pressure (Ptp) was
measured as the difference between the tracheal and intrapleural pressure. The negative intrapleural pressure was detected by a Validyne pressure transducer (Model No. MPX11DP). The entire respiratory system was calibrated with known volume of air and pressure from a water manometer (2 ml or 8 cmH₂O = 2 V output from the pulmonary analyzer). Total pulmonary airway resistance (Rₕ) and dynamic lung compliance (Cdyn) were derived from the input signals of flow and transpulmonary pressure. All measured respiratory parameters were computed on a breath-by-breath basis by a Buxco respiratory analyzer (Buxco Electronics, Sharon, CT), and captured on a microcomputer via a Buxco Model 32 Data Logger. A diagram showing the general experimental set-up is illustrated in Fig. 1.

2.4. Drug administration

Platelet-activating factor (1-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) and bovine serum albumin (BSA) were obtained from Sigma (St Louis, MO). 0.1% BSA in 0.9% NaCl solution was used to dissolve PAF (up to 200 ng/ml), which was infused into the guinea pig through the cannulated external jugular vein. The infusion rate was maintained at 0.1 ml/kg per min by a Sage syringe pump (Model 220, Orion Research, Cambridge, MA). Baseline data acquisition with 0.1% BSA in 0.9% saline solution without PAF was started 1 h after surgery and maintained for 1 h. The same BSA-saline solution with PAF was infused at 5, 10 and 20 ng/kg per min for 2 h (n = 4/dose). A control group of animals (n = 5) was infused with medium without PAF. A PAF antagonist, BN 52021, was provided as an intravenous injectant (20 mg/ml) by Dr. P. Braquet, Institut Henri Beaufour, Le Plessis Robinson, Paris, France. BN 52021 was administered intravenously as a single dose.
TABLE 1

BASELINE VALUES OF CARDIOVASCULAR VARIABLES IN ANESTHETIZED STRAIN 13 GUINEA PIGS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dose of PAF infusion (μg · kg per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>64.2 ± 5.8</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td>237 ± 8.0</td>
</tr>
<tr>
<td>CI (l. min per m²)</td>
<td>1.78 ± 0.4</td>
</tr>
<tr>
<td>TPR (dyn · cm⁻² · s⁻¹ × 10⁻⁵)</td>
<td>42.2 ± 3.4</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>96.3 ± 5.2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>110 ± 4.0</td>
</tr>
<tr>
<td>-dp/dtmax (mmHg · s⁻¹)</td>
<td>20.8 ± 19</td>
</tr>
<tr>
<td>-(dp/dt)/P (s⁻¹)</td>
<td>49.2 ± 2.7</td>
</tr>
<tr>
<td>- dp/dtmin (mmHg · s⁻¹)</td>
<td>1431 ± 151</td>
</tr>
</tbody>
</table>

Mean BP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; TPR, total peripheral resistance; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; - dp/dt, first derivative of the left ventricular pressure (index of cardiac contractility); -(dp/dt)/P, ratio of cardiac contractility index over pressure generated by the heart. - dp/dtmin, cardiac relaxation.

at 6 mg/kg to a separate group of guinea pigs (n = 5) 15 min after starting PAF-infusion (20 ng/kg per min).

2.5. Statistics

All results are expressed as mean ± S.E. Statistical analysis was performed by a repeat ANOVA, and followed by a paired t-test to compare cardiopulmonary responses at various intervals to the baseline (pre-infusion) levels. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Cardiovascular responses to PAF

The baseline values of the hemodynamic variables of anesthetized strain 13 guinea pigs are presented in Table 1. During PAF intravenous infusion at various
Platelet-activating factor induced instantaneous and profound decreases in BP (Fig 2a) and CI (Fig 2b). These hemodynamic responses were evident even after a very small dose of PAF infused (5 ng/kg per min). As PAF doses increased to 10 or 20 ng kg per min, cardiac output was drastically decreased. When CI dropped to less than 300-400 ml/min per m², all the animals died. The 20 ng/kg per min dose of PAF was lethal for all five animals. In guinea pigs surviving the 2 h infusion of lower doses of PAF (5 and 10 ng/kg per min), decreased blood pressure gradually returned to a pre-infusion level within 30-40 min. However, the simultaneously depressed cardiac output remained at low levels with little improvement. As hypotension and low CO were not accompanied by a change in heart rate (Fig 2c), the calculated stroke volume was decreased (data not shown). Another drastic change in hemodynamic responses to PAF infusion was an increased total peripheral resistance (TPR) (Fig 2d).

Within a few minutes after PAF infusion (5 or 10 ng/kg per min), there were profound decreases in LVSP (Fig 3a) and \( +\frac{dp}{dt_{\text{max}}} \) (Fig 3b). The decreased LVSP gradually approached pre-infusion levels (Fig 3a) and depressed \( +\frac{dp}{dt_{\text{max}}} \) (Fig 3b) and \( (\frac{dp}{dt})/P \) (Fig 3c) partially recovered. Under these conditions,
3.2. Effects of BN 52021 on PAF-induced cardiovascular disturbances

When serious hemodynamic disturbances had been established by a potentially lethal PAF infusion (20 ng/kg per min) for 15 min, a single dose (6 mg/kg) of BN
52021 was given i.v., and death was prevented. The decreased BP was elevated to the pre-infusion level within 15 min (Fig 2a). Also, BN 52021 was responsible for a partial recovery of decreased cardiac output (Fig 2b) and $-dP/dt_{max}$ (Fig 4b).

3.3. Pulmonary responses to PAF and antagonism of BN 52021

The baseline levels of pulmonary variables in anesthetized strain 13 guinea pigs are summarized in Table 2. During 2 h infusion of PAF at 5 or 10 ng/kg per min, significant hemodynamic and cardiac response occurred with few changes in respiratory functions. However, in the response to a high dose PAF infusion (20 ng/kg per min), $P_{TP}$ increased (Fig 5a) and $V_T$ decreased (Fig 5b). The most obvious pulmonary responses to PAF at the highest dose were decreases in respiratory frequency (RF, Fig 5c) and minute volume ($V_{E}$) (Fig 5d). A decrease in
TABLE 2  
BASELINE VALUES OF PULMONARY VARIABLES IN ANESTHETIZED STRAIN 13 GUINEA PIGS  

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dose of PAF infusion (ng/kg per min)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>20 + BN 52021</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_t$ (ml)</td>
<td></td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>3.1 ± 0.3</td>
<td>2.8 ± 0.1</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>RF (breath/min)</td>
<td></td>
<td>54.2 ± 3.7</td>
<td>56.7 ± 5.4</td>
<td>64.3 ± 2.7</td>
<td>64.7 ± 3.5</td>
<td>67.4 ± 1.7</td>
</tr>
<tr>
<td>$i$ (ml/min)</td>
<td></td>
<td>131 ± 0.9</td>
<td>129 ± 3.2</td>
<td>134 ± 1.9</td>
<td>133 ± 1.3</td>
<td>136 ± 0.8</td>
</tr>
<tr>
<td>$P_{TP}$ (cmH$_2$O)</td>
<td></td>
<td>4.9 ± 0.6</td>
<td>5.3 ± 0.3</td>
<td>5.2 ± 0.3</td>
<td>6.0 ± 0.8</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>$C_{aw}$ (ml cmH$_2$O/ml per s)</td>
<td></td>
<td>0.49 ± 0.02</td>
<td>0.54 ± 0.01</td>
<td>0.51 ± 0.02</td>
<td>0.55 ± 0.09</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>$C_{aw}$ (cmH$_2$O/ml per s)</td>
<td></td>
<td>0.18 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.20 ± 0.02</td>
</tr>
</tbody>
</table>

$V_t$, tidal volume; RF, respiratory frequency; $V_t$, minute volume; $P_{TP}$, transpulmonary pressure; $C_{aw}$, dynamic lung compliance; $R_t$, pulmonary airway resistance.

Fig. 5. Effects of PAF intravenous infusion on tidal volume ($V_t$; a), transpulmonary pressure ($P_{TP}$; b), respiratory frequency (RF; c) and minute volume ($V_t$; d). See Fig. 2 for illustration.
Fig. 6. Effects of PAF intravenous infusion on dynamic lung compliance ($C_{dyn}$; a) and total pulmonary airway resistance ($R_{1}$; b). See Fig. 2 for illustration.

$C_{dyn}$ was induced at or after 30 min infusion of PAF at 10 or 20 ng/kg per min (Fig. 6a). Increased $R_{1}$ was observed only after PAF infusion at 20 ng/kg per min (Fig. 6b). BN 52021 blocked the lethality of PAF-induced respiratory disturbances through rapid reversal of depressed RF and $V_{E}$ (Fig. 5c and 5d).

3.4. Correlation between PAF-induced cardiovascular and respiratory dysfunction

Fig. 7 shows continuous data acquisition during PAF infusion at 20 ng/kg per min. The sequential changes in cardiopulmonary functions induced by PAF at this lethal dose were: (1) decreases in BP and cardiac functions (LVSP, $dP/dt$, and CI) at 5–10 min; (2) depression of respiratory functions ($V_{E}$, $C_{dyn}$, and RF) and increasing $R_{1}$, accompanied by very low CO; (3) cessation of cardiac pumping...
Fig. 7. Continuous data acquisition of PAF-induced cardiovascular and respiratory disturbances. The cardiopulmonary functions were computed and analyzed during a lethal-dose PAF infusion (20 ng, kg per min). All results are expressed as percent of change from preinfusion levels.

(undetectable CO), and then occurrence of apnea; and (4) cardiac responses (hypertension, high LVSP and dP/dt, cardiac arrhythmia) to ischemia and hypoxia, leading to cardiac arrest.

4. Discussion

The reported cardiovascular responses to in vivo PAF administration include decreases of arterial blood pressure, cardiac output, and myocardial contractility
Although the mechanisms of PAF-induced hypotension were shown to be associated with right ventricular failure and the secondary release of thromboxane A_2 (Laurindo et al., 1989), the hypotensive and adverse cardiac effects of PAF were blocked by intravenous infusion of selected PAF-receptor antagonists, including BN 52021 and SDZ 63-441 (Siren and Feuerstein, 1989). Platelet-activating factor has also been demonstrated to induce bronchial hyper-responsiveness (Chung et al., 1986), broncho-constriction (Nijkamp et al., 1989), inflammatory reaction (Camassi et al., 1983) and airway edema (ODonnell et al., 1985).

In a review of the literature, we noted that PAF-induced cardiovascular and respiratory disturbances were derived mainly from isolated organs and in vivo observations with a single i.v. injection or short duration of PAF infusion (within minutes) (Koltai et al., 1991; Siren and Feuerstein, 1989; Feuerstein et al., 1989; Barnes et al., 1989). In our experimental paradigm, we constantly infused PAF i.v. for 2 h in order to establish the course of PAF-induced cardiopulmonary dysfunctions. The long-term infusion of low doses of PAF may reflect the PAF-mediated pathophysiological events during certain disease states. Furthermore, the simultaneous measurements of PAF-induced cardiovascular and respiratory dysfunctions provide a better understanding of mutual influence of these two vital systems, when blood and tissue PAF concentrations are both increased.

Our data indicate that PAF at lower doses of 5-10 ng/kg per min decreased BP and LVSP. These cardiovascular changes were reversed spontaneously despite continued PAF infusion. Also, the depressed LVSP, +dP/dt\_max and -dP/dt\_max, partially recovered. However, the decreased cardiac output was irreversible during PAF infusion. This PAF-depressed cardiac output might be a result of impaired cardiac contractility, as indicated by decreased +dP/dt\_max, (dP/dt)/P, and decreased plasma volume due to increased capillary permeability (Handley et al., 1985). These results suggest that, in certain cardiovascular diseases, an increased PAF concentration may mainly impair cardiovascular functions and persistently decrease cardiac output.

Felix et al. (1990a) reported that, in anesthetized guinea pigs, single i.v. injection of a large dose (10 μg/kg) of PAF induced left ventricular pump failure, documented by an increase in LVEDP and a drastic decrease in CO and BP. In our experimental paradigm, both +dP/dt\_max and -dP/dt\_max were decreased, but LVEDP was unchanged. The unchanged LVEDP after PAF infusion may be due to reduced left ventricular end diastolic volumes as a result of PAF-induced hemococoncentration (Handley et al., 1985).

Recently, Deavers et al. (1991) observed cardiopulmonary and intravascular changes during the sustained infusion of a high dose of PAF (83 ng/kg per min) in rabbits. The immediate responses to PAF infusion were short periods of right ventricular hypertension, followed by decreases in mean arterial blood pressure, LVSP, +dP/dt\_max, -dP/dt\_max and LVEDP. In another report (Feuerstein et al., 1982), the hypotensive response of rats to PAF was accompanied by an increased
heart rate due to a $\beta$-receptor-mediated sympathetic response. However, in our experiments, guinea pigs failed to show tachycardia. Although the heart rate did not change, the sympathetic tone was indeed augmented, as indicated by an elevation in TPR. A constant infusion of PAF may have a direct negative chronotropic effect, and $\beta$-reflexes are often attenuated in anesthetized animals, thus masking a sympathetic-mediated tachycardia.

In contrast to the rapid and profound cardiovascular responses during PAF infusion, respiratory changes were mild and only occurred at a later phase. Since a bolus injection of PAF has shown marked bronchoconstriction in guinea pigs (Hwang et al., 1988; Nijkamp et al., 1989), this phenomenon may be caused by bronchial tachyphylaxis during PAF infusion at low doses. The PAF-induced bronchial tachyphylaxis was also demonstrated in our separate experiments. Using repeat bolus injection of PAF at 50 ng/kg, we found that PAF-induced bronchoconstriction was also markedly attenuated.

During constant i.v. infusion of PAF, pulmonary mechanical changes in strain 13 guinea pigs were similar to those of rabbits (Deavers et al., 1991). In our studies, major pulmonary perturbation induced by PAF at 20 ng/kg per min included decreased RF, $V_{1}$ and $C_{aw}$, and increased $P_{aw}$ and $R_{L}$. These respiratory changes may be interpreted as follows: (1) PAF induced its pronounced respiratory effects by decreasing RF by acting at the respiratory center of the medulla oblongata of the brain, and (2) respiratory dysfunctions or failure were a result of pulmonary and brain ischemia caused by decreased cardiac output and high TPR. As a vicious cycle, decreased RF, $C_{aw}$ and $V_{1}$ produced oxygen deficiency which further inhibited the cardiac function. It appears that the drastically decreased CO and RF, leading to hypoxia and apnea, are the main causes of PAF-induced death.

BN 52021 is the most extensively studied PAF antagonist, and this PAF antagonist effectively blocks PAF-induced biological actions (Koltai et al., 1991). In our studies, BN 52021 prevented death induced by the highest dose of PAF (20 ng/kg per min) used in this experiment. During PAF infusion, the major benefits produced by BN 52021 included partial restoration of cardiac contractility, increasing CO and prevention of hypventilation by increasing RF and $V_{1}$.

Previously, we demonstrated increased PAF concentrations in the hearts, lungs and brains in Pichinde virus-infected guinea pigs (Qian et al., 1991; Liu et al., 1992). Some similarities between PAF-induced cardiopulmonary dysfunctions and changes of cardiopulmonary function in Pichinde viral diseases have been found (Peters et al., 1987; Liu et al., 1987). Our results suggest that increased PAF concentrations in these specific vital organs of Pichinde virus-infected guinea pigs may be involved in the development of the cardiovascular and respiratory dysfunctions seen during Pichinde virus-induced disease. Our present data, obtained by 2 h of constant PAF infusion, may reflect the involvement of PAF for pathogenesis in certain diseases. Furthermore, our experimental results suggest that the administration of BN 52021 or other potent PAF antagonists may be beneficial for treatment of these diseases (Koltai et al., 1991).
Acknowledgments

The authors thank Ms Jill Neubauer and Specialist Paul Gambell for excellent technical assistance. We also acknowledge the generous gift of BN 52021, a selective PAF receptor antagonist, from Dr P. Braquet, Institut Henri Beaufour, Le Plessis Robinson, Paris, France.

References


