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Pathophysiological mechanisms of sudden death induced by platelet activating factor

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1 Platelet activating factor (Paf) (15–40 μg^{-1}) kills male rabbits within 3 to 5 min. Intravenous injection of Paf at a dose of 15 $\mu g\,kg^{-1}$ is uniformly lethal, and the rabbits died within 4.5 ± 0.4 min.

2 The sudden death is characterized by cessation of respiration, a marked decrease in mean arterial blood pressure (M.A.B.P.), and 8 fold increases in plasma thromboxane B_2 (TxB_2) concentrations with only modest elevation in plasma 6 keto-prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) concentrations.

3 Pretreatment with the cyclo-oxygenase inhibitor, ibuprofen (6.25 mg kg^{-1}), or with the thromboxane synthetase inhibitors dazoxiben (2.5 mg kg^{-1}), CGS-13080, or OKY-046 1 mg kg^{-1}) increased survival rates to 83–100%.

4 Protected rabbits showed only modest changes in M.A.B.P. and no significant increase in plasma TxB_2 concentrations. The protective drugs showed a dose-related action on M.A.B.P., plasma TxB_2 concentration and mortality rate in Paf-induced sudden death.

5 The mechanisms of the protection appeared to be prevention of platelet aggregation (leading to pulmonary thrombosis) and pulmonary and coronary vasoconstriction. However, Paf does not appear to exert direct vasoconstrictor effects in isolated coronary or pulmonary arteries.

6 The effects of Paf *in vivo* appear to be mediated by TxA_2 released by activated platelets in the absence of the protective effects of prostacyclin. Inhibition of thromboxane synthesis effectively prevents the Paf-induced sudden death.

Introduction

Platelet activating factor (Paf), a low molecular weight phospholipid, is known to be released from various cell types after an immunological challenge (Benveniste *et al.*, 1972). Specifically, Paf is released from rabbit platelets and basophils, mouse macrophages, and human polymorphonuclear neutrophils in the presence of specific secretagogue stimuli such as thrombin, zymosan, complement coated zymosan and the calcium ionophore A23187 (Benveniste *et al.*, 1982). The molecular structure of Paf has been identified as 1-0-alkyl-2-acetyl-sn-glycerophosphorylcholine (Demopoulos *et al.*, 1979). *In vitro*, Paf has been shown to have a strong dose-dependent platelet aggregatory activity, and additionally releases 5-hydroxytryptamine and platelet factor 4 from platelet granules (Chignard *et al.*, 1979; Chesney *et al.*, 1982; Fouque *et al.*, 1982). Paf also activates neutrophils leading to the release of free radicals (e.g., superoxides) and lysosomal hydrolases (Marche *et al.*, 1982).

In the isolated perfused lung of the rabbit as well as in the anaesthetized rabbit, Paf induces dose-dependent increases in intravascular concentrations of thromboxane A_2 (TxA_2) (McManus *et al.*, 1983). TxA_2 is a potent vasoconstrictor and platelet aggregator formed from arachidonic acid and can be released either by Paf stimulated platelets or by the infusion of Paf (McManus *et al.*, 1983; Heffner *et al.*, 1983). This release of TxA_2 was accompanied by the development of thrombocytopaenia, neutropaenia, pulmonary hypertension and increased vascular permeability, and is similar to the thrombocytopaenia and platelet-dependent bronchoconstriction observed in the guinea-pig (Chignard *et al.*, 1982) upon Paf challenge.

When Paf was infused intravenously in dogs, it induced shock resulting in a dose-related fall of systemic blood pressure and cardiac output without changes in heart rate, plasma volume or pulmonary

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arterial pressure (Bessin *et al.*, 1983). Higher doses of Paf reduced coronary blood flow, diminished myocardial O_2 consumption and caused metabolic acidosis leading to death. These changes are similar to those occurring in acute circulatory collapse due to hypovolemia (Bessin *et al.*, 1983). In rats, comparable haemodynamic changes occurred (Caillard *et al.*, 1982). Several agents such as thromboxane synthetase inhibitors (Heffner *et al.*, 1983), TxA_2 antagonists (Heffner *et al.*, 1983), cyclo-oxygenase inhibitors (Chignard *et al.*, 1982), metabolic inhibitors, and membrane active drugs (Chesney *et al.*, 1982) when used *in vitro* have been found to reduce the aggregation and secretion of platelets (Chesney *et al.*, 1982), diminish pulmonary damage (Heffner *et al.*, 1983), and reduce the increase of TxA_2 produced by Paf.

The purposes of this study were (a) to determine the minimal lethal dose of Paf required to produce sudden death uniformly in rabbits and assess its mechanism of inducing death and, (b) to compare the protective effects of a non-steroidal anti-inflammatory cyclo-oxygenase inhibitor (e.g., ibuprofen) along with specific thromboxane synthetase inhibitors *in vivo* in this sudden death model.

Methods

Forty-four adult male New Zealand rabbits weighing between 2.5–3.9 kg were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) injected intravenously. A tracheal cannula was connected to a Statham differential pressure transducer for the recording of intratracheal pressure. Polyethylene catheters were inserted into the right femoral artery to monitor mean arterial blood pressure (M.A.B.P.) and into the right and left femoral vein for the injection of Paf and the appropriate vehicles or drugs. A scalar electrocardiogram employing Lead III was recorded on a Beckman Model R411 oscillographic recorder. Paf (15 $\mu g\,kg^{-1}$) freshly diluted in bovine albumin (1 mg ml^{-1} in 0.9% NaCl) were injected over 50–60 s. The drugs or their vehicles, ibuprofen (6.25 mg kg^{-1} in 0.9% NaCl), dazoxiben (2.5 mg kg^{-1} in 0.9% NaCl), OKY-046 (Sodium (E)-3-[4-(1 imidazolyl methyl) phenyl] -2-propanoate, 1 mg kg^{-1} in 0.9% NaCl), and CGS-13080 (imidazo (1,5-a) pyridine-5-hexanoic acid, 2.5 mg kg^{-1} in 0.01 M Tris buffer) were injected intravenously 15 min prior to the administration of Paf.

Blood samples (3 ml) were with drawn 1 min before drug injection, 1 min before Paf injection, and 15 min after the injection of Paf or just prior to death. Blood samples were drawn into 30 μl disodium edetate (EDTA, 50 mM) containing 120 units heparin. The samples were centrifuged at 6500 *g* at 4°C for 10 min. The plasma was decanted and frozen until assayed for thromboxane B_2 (TxB_2), the stable metabolite of TxB_2, by a specific radioimmunoassay according to the method previously described by Lewy *et al.* (1979). 6-keto Prostaglandin $F_{1\alpha}$, a metabolite of PGI_2 (prostacyclin) was measured with a specific radioimmunoassay described by Smith *et al.*, (1980). Postmortem examination of the lungs from the Paf-injected rabbits was performed. The lungs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin, and photomicrographs taken at 200 to 450 × magnification.

Pulmonary arteries were obtained from adult male rabbits anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). Spirally cut artery strips were prepared according to the method of Smith *et al.* (1981), suspended in 20 ml muscle chambers. Dimensions of the prepared vessel strips were 15–20 mm × 3–4 mm. Arterial strips were bathed in oxygenated (95% O_2 + 5% CO_2) Krebs-Henseleit solution warmed to 37°C at a resting force of 1 g and allowed to equilibrate for 2 h under these conditions before administering any agent. Isometric contractions were recorded on a Grass Model 7 oscillographic recorder using Grass FT-03 force transducers. Fresh Krebs-Henseleit (K-H) solution was introduced into the bath periodically during the equilibration period and following each test response. Pulmonary artery strips were given noradrenaline 100 ng ml^{-1} to test vascular smooth muscle responsiveness. The noradrenaline was washed out and either 0.1, 1, or 10 $\mu g\,ml^{-1}$ Paf was added to the bath. After 10–15 min, the Paf was washed out and the test dose of noradrenaline was repeated.

Results

The method of Paf-induced sudden death employed in this present study generally resulted in a dose-dependent mortality of anaesthetized rabbits. Doses of 10 $\mu g\,kg^{-1}$ Paf failed to induce death. However, 15 $\mu g\,kg^{-1}$ Paf had a uniformly lethal effect, and 12 rabbits given only the vehicle for the tested drugs all died. In contrast, in the drug-treated group the survival was 100% following the cyclo-oxygenase inhibitor ibuprofen (6.25 mg kg^{-1}, the selective thromboxane synthetase inhibitors dazoxiben (2.5 mg kg^{-1}) and OKY-046 (1 mg kg^{-1}), and 83% when the rabbits were pretreated with the CGS-13080 (2.5 mg kg^{-1}). Lower doses of these protective agents failed to achieve high survival rates. Table 1 summarizes these results.

The sudden death induced by Paf was characterized by a marked decrease in mean arterial blood pressure (M.A.B.P.) within 4 min. This hypotension was initially associated with a bronchoconstriction and a cessation of respiration about 1 min after infu-

Table 1 Effects of thromboxane synthetase and cyclo-oxygenase inhibitors on platelet activating factor (Paf)-induced sudden death

Drug/Veh	Dose of test drug ($mg\,kg^{-1}$)	Paf ($\mu g\,kg^{-1}$)	*Total number of rabbits*	*Number of survivors*	*% survival*	*Significance* (χ^2)
Vehicle	-	15	12	0	0	-
Ibuprofen	6.25	15	5	5	100	$P<0.001$
Dazoxiben	2.5	15	5	5	100	$P<0.001$
OKY-046	1.0	15	5	5	100	$P<0.001$
CGS-13080	2.5	15	6	5	83	$P<0.01$

sion of Paf (See Figure 1). The initial M.A.B.P. was not significantly different among any of the 5 groups of rabbits studied. However, M.A.B.P. fell markedly by 3 min after the administration of Paf ($P<0.001$) in all groups of rabbits as shown in Figure 2. M.A.B.P. decreased dramatically in Paf-treated rabbits falling to values approaching 0 mmHg in about 5 min. All drug-treated groups exhibited a much less severe decline in M.A.B.P., falling to about 40–60 mmHg at 3 min, and increasing gradually to about 70–75 mmHg at 15 min. By this time, the rabbits receiving Paf and only the vehicle for one of the inhibitors had already died, as shown in Figure 2.

Figure 3 summarizes the plasma TxB_2 changes observed in response to 15 $\mu g\,kg^{-1}$ Paf. The concentration of TxB_2 was initially very low in all groups of rabbits.

However, in those rabbits receiving only the vehicle for the drugs (0.9% NaCl, or 0.01 M Tris buffer), the final plasma TxB_2 value increased more than 8 fold to 9.7 ± 1.8 pmol ml^{-1} compared to pre-Paf values indicating that at 3–5 min when these samples were drawn, a large amount of thromboxane A_2 is formed. These increases in TxB_2 occurred during the period when the lethal events responsible for the death such as platelet aggregation, bronchoconstriction, pulmonary thrombosis, and respiratory arrest are most prominent (Figure 4). In addition to TxB_2, plasma concentrations of 6-keto $PGF_{1\alpha}$, the stable metabolite of PGI_2, was observed in the untreated rabbits.We observed an initial 6-keto $PGF_{1\alpha}$ concentration of 0.7 ± 0.2 pmol ml^{-1}. These values increased after Paf administration to 4.0 ± 0.8 pmol ml^{-1} ($P<0.005$) but these values are only modestly increased compared with the increases in thromboxane B_2 concentrations.

When the doses of the cyclo-oxygenase inhibitor ibuprofen and the thromboxane synthetase inhibitors dazoxiben, OKY-046 (OKY), and CGS-13080 (CGS) were injected, there was a marked inhibition in the increases in TxB_2 concentrations in response to Paf. In contrast to the untreated rabbits, these rabbits exhibited only a slight depression of the cardiovascular and pulmonary system and the TxB_2 plasma levels were not statistically changed (Figure 3). However, when lower concentrations of these drugs were employed (i.e., 1.6, 3.1 $mg\,kg^{-1}$, ibuprofen; 1.0 $mg\,kg^{-1}$ dazoxiben and 1 $mg\,kg^{-1}$ CGS-13080) they failed to prevent increases in TxB_2 plasma concentrations and did not prevent sudden death. Thus, the cyclo-oxygenase and thromboxane synthetase inhibitors described in this study prevent the Paf-induced sudden death model concomitant with the inhibition of TxB_2 generation.

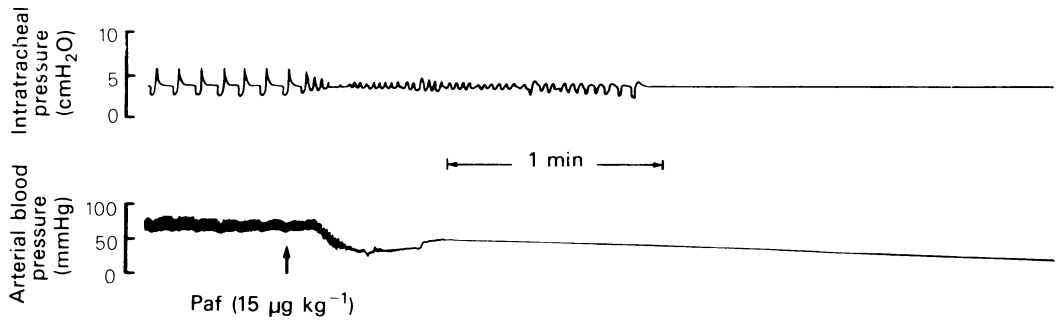

Figure 1 Platelet activating factor (Paf, 15 $\mu g\,kg^{-1}$) induced sudden death with decreasing M.A.B.P. initially associated with bronchoconstriction and a cessation of respiration. Death occurred within 3 min.

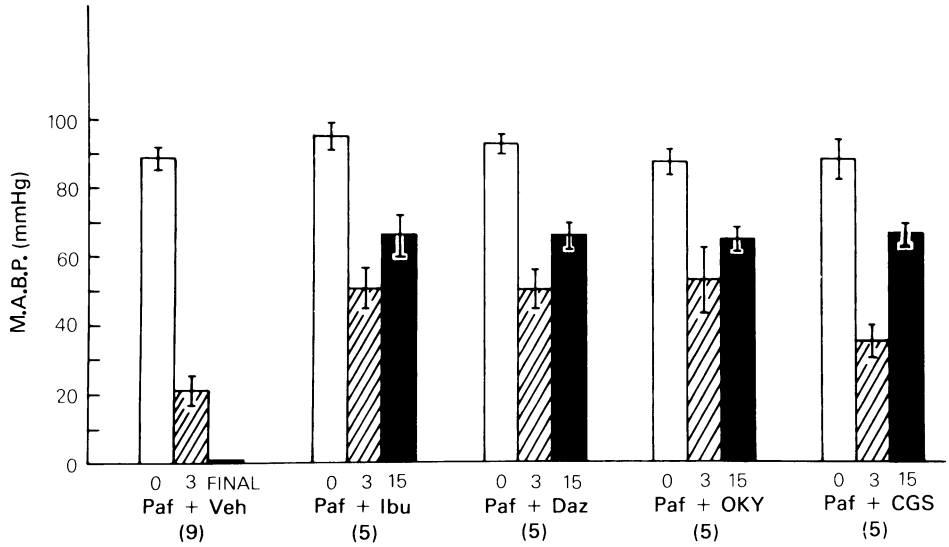

Figure 2 Mean decrease in M.A.B.P. (mm Hg) 0, 3, final (3–5 min) after the challenge with platelet activating factor (Paf). Ibuprofen (Ibu) dazoxiben (Daz) OKY-046 (OKY) and CGS-13080 are the protective drugs used. Vertical lines indicate s.e.mean. Numbers in parentheses are numbers of rabbits in each group.

Paf, at concentrations of 0.1 to 10 $\mu g\,ml^{-1}$, exerted no detectable direct vasoactive effects in rabbit pulmonary artery strips. Table 2 summarizes the typical responses to Paf and noradrenaline. Noradrenaline at 100 $ng\,ml^{-1}$ produced a marked contraction of the strip. The average initial force of contraction to noradrenaline was between 840 and 862 mg. However, Paf at 0.1, 1, or 10 $\mu g\,ml^{-1}$ failed to contract the

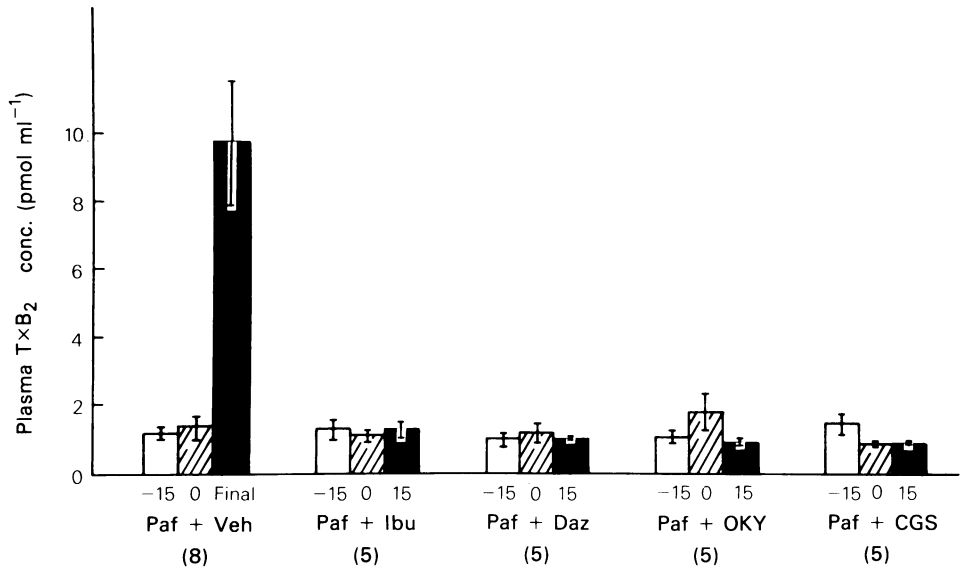

Figure 3 Mean plasma thromboxane B_2 concentrations expressed in pmol ml^{-1}. -15 = 1 min before vehicle or drug and 15 min before Paf injection (0). 0 = 1 min before Paf challenge. Final = before death, about 4 min after Paf (changes to −15 are highly significant, $P<0.001$). 15 = 15 min after Paf challenge with the rabbit still alive (no significance to −15, $P<0.05$). Vertical lines indicate s.e.mean. Numbers in parentheses are numbers of rabbits in each group.

Table 2 Vascular responses of rabbit pulmonary artery to platelet activating factor (Paf)

	Developed force (mg)		
Concentration of Paf ($1\,\mu g\,ml^{-1}$)	*NA* ($100\,ng\,ml^{-1}$)	*Paf*	*NA* ($100\,mg\,ml^{-1}$)
0.1	862 ± 103	12 ± 25	858 ± 120
1	840 ± 95	11 ± 20	865 ± 86
10	855 ± 92	8 ± 16	840 ± 81

All values are means ± s.e.mean for 4 to 5 pulmonary artery strips. NA = noradrenaline.

artery strips significantly . In these pulmonary artery strips, Paf increased force by between 8 and 12 mg. These small responses were not statistically significant. Moreover, Paf did not alter the responsiveness to subsequent addition of noradrenaline. None of the second responses to noradrenaline were significantly different from the first noradrenaline response. In four cat coronary arteries, Paf at 0.1 to $10\,\mu g\,ml^{-1}$ also failed to exert any vasoactive effects.

Discussion

We have observed that relatively low doses of Paf produce a uniformly lethal form of sudden death in rabbits. The sudden death is characterized by bronchoconstriction and pulmonary thrombosis, and a precipitous decrease in arterial blood pressure within 3 min of intravenous injection of Paf. Evidence of myocardial ischaemia or coronary insufficiency (e.g., S-T segment elevation, large broad T-waves) was also observed at this time. When these disturbances of cardiopulmonary function occurred, death followed in about 3 to 5 min. The biological profile is quite similar to the sudden death produced by intravenous injection of arachidonic acid ($2\,mg\,kg^{-1}$) in rabbits (Lefer *et al.*, 1981; Roth *et al.*, 1983).

In arachidonic acid-induced sudden death, the primary mediator of the sudden death is thromboxane A_2 (Smith *et al.*, 1980). The data supporting this conclusion are (a) circulating thromboxane A_2 concentrations increase about 10 fold just before death, (b) thromboxane synthetase inhibitors prevent thromboxane formation and protect against sudden death (Randell & Parry, 1981; Lefer *et al.*, 1981), (c) thromboxane-like analogues (i.e., carbacyclic thromboxane A_2, 9, 11-azo PGH_2 and 9, 11 methanoepoxy PGH_2) mimic the sudden death induced by arachidonic acid (Smith *et al.*, 1981; Lefer *et al.*, 1983; Myers *et al.*, 1983) and (d) all of the major pathophysiological events leading to the sudden death (i.e., platelet aggregation, pulmonary thrombosis, coronary vasoconstriction) are produced by thromboxane A_2 (Terashita *et al.*, 1978; Smith *et al.*, 1980; Yamazuki *et al.*, 1983).

Our attempts to prevent thromboxane generation in response to Paf injection by use of cyclo-oxygenase or thromboxane synthetase inhibitors were successful. Thus, we were also able to duplicate the protective profile of pharmacological agents in arachidonic acid-induced sudden death. Our results are also consistent with a primary mediator role of thromboxane A_2 in Paf-induced sudden death. Paf appears to exert specific effects on platelets resulting in the massive activation and rapid aggregation (Chignard *et al.*, 1979; Chesney *et al.*, 1982). A significant aspect of the actions of Paf on platelets appears to be rapid synthesis and release of thromboxane A_2 by the activated platelets (Heffner *et al.*, 1983). Since intravenous injection of Paf presumably produces this platelet release of thromboxane A_2 and platelet aggregation rapidly, these microthrombi and the circulating plasma rich in thromboxanes will travel rapidly to the lungs where they will exert pulmonary artery constriction and pulmonary vascular thrombosis. As some of the circulating thromboxane passes through the lungs, it will circulate to the heart where coronary constriction will rapidly occur. Since we have shown that Paf does not produce coronary or pulmonary artery constriction directly, the vascular effects of Paf are probably mediated by thromboxanes in this model of sudden death.

This form of sudden death is different from arachidonic acid-induced sudden death with regard to prostacyclin (PGI_2) concentrations. Circulating PGI_2 levels are not markedly elevated in Paf-induced sudden death in comparison to the dramatic elevation in PGI_2 concentrations produced by arachidonic acid (Smith *et al.*, 1980; Yamazuki *et al.*, 1983). PGI_2 does not play a significant role in the pathogenesis of arachidonic acid-induced sudden death since it is elevated in the presence of arachidonate plus thromboxane synthetase inhibitors despite the total survival in those animals (Smith *et al.*, 1980; Yamazuki *et al.*, 1983). In Paf-induced sudden death, moderate amounts of PGI_2 are generated because the stimulus is more specific for platelets than that produced by arachidonic acid which also stimulates vascular tissue to produce a variety of prostaglandins including PGI_2. Of course, PGI_2 exerts effects opposite to those of thromboxane A_2 which, would be of protective value in hypoxic disorders (Araki & Lefer, 1980).

Presumably, agents other than cyclo-oxygenase or thromboxane synthetase inhibitors that interfere with the formation, circulation or action of thromboxane A_2 (e.g., calcium channel blockers, thromboxane receptor antagonists) could protect against Paf-induced sudden death as they do in arachidonic

acid-induced sudden death (Bonnet *et al.*, 1981; Okamatsu *et al.*, 1981; Heffner *et al.*, 1983). Since a variety of immunological and chemical stimuli are known to result in activation or release of Paf in circulating blood, Paf-induced sudden death may be a real phenomenon in animals or man. In this regard, Paf has been found to produce a spontaneous form of circulatory shock (Bessin *et al.*, 1983), not dissimilar from that observed in endotoxic shock.

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