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Effects of a pyridine derivative thromboxane synthetase inhibitor and its inactive isomer in endotoxic shock in the rat

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1 We investigated the effects of a pyridine derivative thromboxane synthetase inhibitor and its inactive *ortho* isomer on arachidonic acid metabolism and pathophysiological sequelae of endotoxic shock. In vehicle-treated rats, 30 min after intravenous *S. enteritidis* endotoxin (15 mg/kg), plasma $iTxB_2$ (the stable metabolite of thromboxane A_2) increased from non-detectable levels (< 100 pg/ml) to 763 ± 250 pg/ml ($n = 10$). Plasma i6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin, PGI_2) increased to 1850 ± 426 pg/ml, ($n = 9$) and plasma iPGE increased to $2350 = 560$ ($n = 5$). Pretreatment with the pyridine active (PA) *meta* isomer (30 mg/kg i.p.) significantly ($P < 0.05$) suppressed $iTxB_2$ to 390 ± 31 pg/ml ($n = 10$) although 6-keto-$PGF_{1\alpha}$ levels (1294 ± 358 pg/ml, $n = 5$) and plasma iPGE (2847 ± 1103 pg/ml, $n = 5$) were not significantly different from the shocked control values. In contrast, pretreatment with, the pyridine inactive (PI) *ortho* isomer did not significantly affect endotoxin-induced $iTxB_2$ (1431 ± 194 pg/ml, $n = 5$) or i6-keto-$PGF_{1\alpha}$ synthesis (628 ± 266 pg/ml, $n = 5$).

2 Pretreatment of rats with the Tx synthetase inhibitor, PA, significantly enhanced ($P < 0.05$) survival and prevented splanchnic infarction relative to both endotoxin shocked control rats and those pretreated with the PI isomer.

3 Significantly reduced lysosomal labilization, hepatocellular dysfunction and elevations in serum fibrin/fibrinogen degradation products were seen only in groups pretreated with the PA isomer.

4 The beneficial effects of the latter compound in endotoxic shock thus appear to be due to inhibition of Tx synthesis, since its *ortho* isomer did not inhibit TxA_2 synthesis nor did it protect against endotoxic shock.

Introduction

The participation of arachidonic acid metabolites in experimental endotoxic and septic shock is suggested from previous investigations (Parratt & Sturgess 1975; Fletcher & Ramwell 1980a; Rietschel, Schade, Luderitz, Fischer & Peskar 1980; Bult & Herman 1982). Recent studies have demonstrated increased plasma thromboxane $(Tx)B_2$, the stable metabolite of TxA_2, in response to endotoxin (Cook, Wise & Halushka 1980; Frolich Ogletree, Peskar & Brigham 1980; Harris, Zmudka, Maddox, Ramwell & Fletcher 1980; Demling, Smith, Gunther, Flynn & Gee 1981; Coker, Hughes, Parratt, Rodgers & Zeilin 1981). TxA_2 is a potent vasoconstrictor and pro-aggregatory substance synthesized by a variety of cells including platelets (Moncada & Vane, 1979) and macrophages (Cook, Wise, Knapp & Halushka, 1981a; Feuerstein, Foegh & Ramwell, 1981), which are target cells for endotoxin. The Tx synthetase inhibitors, imidazole (Cook *et al.*, 1980; Smith, Tabas & Lefer 1980; Watkins, Huttemeier, Kong & Peterson, 1982); 7-(1-imidazolyl) heptanoic acid (Wise, Cook, Knapp & Halushka, 1980a), and UK37,248 (Halushka, Cook & Wise, 1982) have been found to improve survival significantly and/or reduce the sequelae from endotoxaemia. Therefore it would appear that TxA_2 may be a deleterious factor in endotoxic shock.

While these Tx synthetase inhibitors are protective

in endotoxic shock, they are imidazole based compounds and may exhibit salutary actions in endotoxic shock independent of inhibition of Tx synthetase. For example, imidazole stimulates phosphodiesterase, stabilizes lysosomal membranes, and can affect Ca^{2+} fluxes (Puig-Muset, Puig-Parallada & Martin-Esteve, 1972). Thus, it is important to test the protective efficacy of other Tx synthetase inhibitors that are not analogues of imidazole.

We have recently observed that a series of pyridine compounds synthesized by the Dow-Merrell Pharmaceutical Company are effective inhibitors of Tx synthetase. Of particular interest are two compounds: 4-((3-pyridinylmethyl) amino) benzoic acid (PA), and 4-((2-pryidinylmethyl) amino) benzoic acid (PI) (Figure 1). The active Tx synthetase inhibitor, PA, has an alkyl side chain substituted with the nitrogen in the *meta* position whereas the *ortho* isomer, PI, is not an effective inhibitor of Tx synthetase. The inactive pyridine derivative therefore provided a control for potential effects of this chemical structure unrelated to inhibition of thromboxane synthesis. The present studies were undertaken to assess the effect of these two compounds on the pathophysiological events of endotoxic shock in the rat. Specifically we evaluated the effects of these compounds on (1) endotoxin induced *in vivo* and *in vitro* synthesis of immunoreactive (i) TxB_2 and i6-keto-$PGF_{1\alpha}$ (the stable immunoreactive metabolite of PGI_2); (2) mortality; (3) splanchnic infarction; (4) lysosomal labilization; (5) hepatic dysfunction and (6) serum fibrin/fibrinogen degradation products.

Methods

Male Long-Evans rats, weighing 180–230 g were used for these experiments; they were chosen from a breeding colony maintained by the investigators. Rats were housed in plastic cages with 'Beta Chip' hardwood bedding under conditions of constant temperature and controlled illumination. Rat food was obtained from Wayne Laboratories, Allied Mills, Inc. (Chicago, Ill.) and contained 24% protein, 4.5% fibre and 4.0% fat. Rats were allowed food and water *ad libitum*.

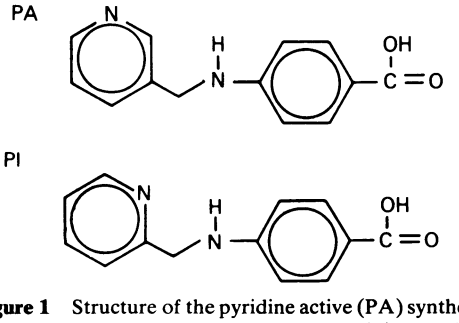

Figure 1 Structure of the pyridine active (PA) synthetase inhibitor with a *meta* substituted pyridinylmethyl amino benzoic acid. The *ortho* isomer of this compound (PI) is an inactive thromboxane synthetase inhibitor.

Endotoxic shock was induced by the intravenous administration, into the dorsal vein of the penis during light ether anaesthesia, of *Salmonella enteritidis* endotoxin (15 mg/kg). In the mortality studies the rats received 12.5 mg/kg of endotoxin. The endotoxin was a Boivin preparation No.683702 from Difco, Control Laboratories (Detroit, Michigan).

Solutions were prepared by dissolving the pyridine derivatives in sterile saline with 20% 0.1 N NaOH. Rats were given the compound intraperitoneally, 30 min before challenge with endotoxin. Control rats were pretreated intraperitoneally with an isovolumetric dose of 1 ml/100 g body weight.

Radioimmunoassay of immunoreactive (i) $iTxB_2$ and i6-keto-$PGF_{1\alpha}$ and iPGE

During ether anaesthesia the abdominal cavity was opened and blood was obtained from the inferior vena cava for measurement of iPGE or TxA_2 and PGI_2, via their stable metabolites $iTxB_2$ and i6-keto-$PGF_{1\alpha}$, respectively. Blood was collected in plastic syringes containing 0.1 ml of a solution of indomethacin (2 mg/ml) dissolved in 0.1 ml sodium phosphate buffer, pH 8.0, and 500 units heparin. The blood was centrifuged (1500 *g*) for 20 min and the plasma was collected and frozen at −20°C until extracted with ethyl acetate. The extraction, chromatography and radioimmunoassay of iPGE, $iTxB_2$ and i6-keto-$PGF_{1\alpha}$ were as previously described (Burch, Knapp & Halushka 1979; Wise, Cook, Eller & Halushka, 1980b). The minimal detectable amount of $iTxB_2$, iPGE or i6-keto-$PGF_{1\alpha}$ in 1 ml of plasma was approximately 100 pg.

Rat adherent peritoneal macrophages

Male Long-Evans rats were used as a source of peritoneal leukocytes in these studies. Resident peritoneal leukocytes were obtained by peritoneal lavage with 10 ml of sterile RPMI 1640 (Microbiological Associates) containing 50 units of penicillin and 50 μg of streptomycin per ml. Adherent peritoneal cells were obtained by incubating 4 ml of 1×10^6 cells/ml in 60 × 15 mm plastic culture dishes (Falcon) in RPMI 1640 for 2 h. The plates were then washed 3 times with 3 ml of RPMI 1640 medium to remove nonadherent cells. RPMI medium (4 ml) was added to the plates and they were incubated for 5 h.

The pyridine derivatives were dissolved in 1N NaOH and appropriate dilutions were added to RPMI 1640 to give a concentration of 150 or 500 μM.

Isovolumetric doses of the vehicle were added to control cultures. The final pH of the media was adjusted to 7.4 with 0.1N HCL.

After a 5 h incubation the culture medium was collected and assayed directly for $iTxB_2$ and i6-keto-$PGF_{1\alpha}$ using aliquots of different volumes. We have previously found that cellular metabolic products or the unincubated culture media do not significantly alter the radioimmunoassay (Cook, *et al.*, 1981a).

Enzyme assays and fibrin/fibrinogen degradation products

Four hours after administration of endotoxin, the rats were anaesthetized with ether and blood was obtained from the inferior vena cava for determination of the following serum constituents. Total serum acid phosphatase activity was assayed by a modification of the method of Anderesch & Szcypinski (1947). Acid phosphatase activity was determined colorimetrically from the substrate of *p*-nitrophenol-phosphate, one unit of enzyme activity being defined as the amount of enzyme activity that will liberate 1 μmol of *p*-nitrophenol per hour at 37°C. Serum β-glucuronidase activity was assayed by a modification of the procedure of Fishman, Kato, Antess & Green (1967). One unit of enzyme activity was defined as the amount of enzyme that will liberate 1 μg of phenolphthalein from phenolphthalein glucuronate per hour at 56°C. The measurement of fibrinogen and fibrin degradation products in serum was based on the method of Hawiger, Niearowski, Gurewich & Thomas (1970) using a commercially available kit (Sigma Chemical, 850). An estimate of the fibrinogen/fibrin degradation products (FDP) present was obtained by comparing the degree of cell staphylococcal clumping produced by the test serum with clumping observed using known amounts of fibrinogen. Plasma glutamic-oxaloacetic and glutamic pyruvic transaminases were measured colorimeterically (Sigma Chemical Bullentin No 505). Activity is expressed as Sigma Frankel units. One unit will form glutamate, 4.8×10^{-4} mol/min, at pH 7.5 and 25°C. Blood glucose was determined by the glucose oxidase test according to Sigma Technical Bullentin No. 510.

Materials

The following were purchased from commercial sources; spectral grade solvents, Burdick-Jackson, Muskegon, MI, [5,6,8,9,11,12,14,15^{3}H]-TxB_2 [60–70 Ci/mM], [5,6,8,11,12,14,15^{3}H] PGE_2 (130 Ci/mM) and [5,8,9,11,12,14,15–^{3}H]-6-keto-$PGF_{1\alpha}$ (100 Ci/mM) New England Nuclear Corp., Boston, Mass. Authentic 6-keto-$PGF_{1\alpha}$, TxB_2 and PGE_2 were kindly provided by Dr John Pike, Upjohn Company, Kalamazoo, Michigan.

Statistical analysis

Student's *t* test for unpaired observations was used to determine differences between untreated and treated groups. The Chi square test was employed for mortality studies. All data are expressed as the mean ± s.e.mean.

Results

Plasma and macrophage $iTxB_2$, i6-keto-$PGF_{1\alpha}$ and iPGE

In vehicle-treated rats within 30 min after endotoxin administration, plasma $iTxB_2$ was 763 ± 250 pg/ml ($n = 10$), plasma i6-keto-$PGF_{1\alpha}$ was 1850 ± 426 pg/ml ($n = 9$) and plasma iPGE was 2350 ± 560 ($n = 5$) (Table 1). Pretreatment with the pyridine active (PA) *meta* isomer suppressed ($P < 0.05$) the endotoxin-induced increase in plasma $iTxB_2$ to 139 ± 31 pg/ml ($n = 10$), while plasma i6-

Table 1 Effect of the pyridine active (PA) and inactive (PI) derivative on plasma, $iTxB_2$, i6-keto-$PGF_{1\alpha}$ and iPGE 30 min after endotoxin (LPS)

Group	*$iTxB_2$* (pg/ml)	*i6-keto-$PGF_{1\alpha}$* (pg/ml)	*iPGE* (pg/ml)
Vehicle + LPS	763 ± 250 (10)	1850 ± 426 (9)	2350 ± 560 (5)
PA + LPS	139 ± 31* (10)	1294 ± 358 (5)	2847 ± 1103 (5)
PI + LPS	1431 ± 194 (5)	628 ± 266 (5)	

Compound PA and PI were administered i.p. (30 mg/kg) 30 min before LPS (15 mg/kg). *n* in parentheses.
$iTxB_2$ levels in rats pretreated with compound, PA, without LPS were 68.5 ± 8.5 pg/ml ($n = 4$) and 110 ± 2.7 pg/ml ($n = 4$) for rats pretreated only with PI.
*$P < 0.05$ compared to vehicle + LPS group.

Table 2 Effect of the pyridine active (PA) and inactive (PI) derivative on plasma i6-keto-$PGF_{1\alpha}$ 4 h after endotoxin (LPS)

Group	*i6-keto-$PGF_{1\alpha}$* (pg/ml)
Vehicle + LPS (5)	7,973 ± 733
PA + LPS (5)	2,409 ± 389*
PI + LPS (5)	6,929 ± 1,227

Compound PA and PI were administered i.p. (30 mg/kg) 30 min before LPS (15 mg/kg). *n* in parentheses.
*$P < 0.001$ compared to vehicle + LPS and the PI group.

keto-$PGF_{1\alpha}$ (1294 ± 358 pg/ml, $n = 5$) and plasma PGE (2847 ± 1103 pg/ml, $n = 5$) did not vary significantly from the shocked vehicle-treated rats. In rats pretreated with the pyridine inactive (PI) *ortho* isomer, endotoxin enhanced $iTxB_2$ levels to 1431 ± 194 pg/ml ($n = 5$) and plasma i6-keto-$PGF_{1\alpha}$ levels to 628 ± 266 pg/ml ($n = 5$). The latter values were not significantly different from shocked vehicle-treated controls. Both of the isomers also were injected into non-shocked control rats. Rats pretreated with the PI derivative exhibited slight but significantly ($P < 0.05$) elevated $iTxB_2$ levels (110 ± 2.7 pg/ml) ($n = 4$) relative to the group pretreated with the PA derivative (68.5 ± 8.5 pg/ml) ($n = 4$).

Since we have previously observed that i6-keto-$PGF_{1\alpha}$ levels continue to rise in the delayed shock phase, plasma levels of this metabolite were measured 4 h after endotoxin (Table 2). In shocked vehicle-treated rats, plasma i6-keto-$PGF_{1\alpha}$ levels were 7973 ± 733 pg/ml ($n = 5$) and were not significantly different from rats pretreated with the PI derivative (6929 ± 1227 pg/ml, $n = 5$). However, pretreatment with the *meta* isomer, PA, significantly ($P < 0.001$) decreased plasma i6-keto-$PGF_{1\alpha}$ levels to 2409 ± 389 pg/ml ($n = 5$).

To confirm that the PA compound was a selective synthetase inhibitor, studies were conducted *in vitro* with peritoneal macrophages. Control culture medium after 5 h of incubation contained 11.1 ± 1.1 ng/ml of $iTxB_2$ and 17.6 ± 1.2 ng/ml of i6-keto-$PGF_{1\alpha}$ (Table 3). In the presence of the PA derivative at 150 μM or 500 μM concentrations, $iTxB_2$ levels were reduced ($P < 0.01$) to 3.0 ± 0.2 ng/ml ($n = 3$) and 0.5 ± 0.2 ng/ml ($n = 4$) respectively. There was significant ($P < 0.01$) shunting to i6-keto-$PGF_{1\alpha}$ synthesis at both concentrations. The *ortho* isomer, PI, had no significant effect on $iTxB_2$ or i6-keto-$PGF_{1\alpha}$ synthesis at the 150 μM or 500 μM concentration.

Mortality and splanchnic infarction

Pretreatment with the PA derivative significantly ($P < 0.05$) enhanced survival (60%) ($n = 50$) from endotoxic shock up to 24 h as opposed to 38% ($n = 42$) survival in vehicle-treated rats (Figure 2). Survival of rats treated with the PI compound was only 24% ($n = 33$) and not significantly different from shocked controls.

Splanchnic infarction and haemorrhage was a significant early lesion in most of the vehicle-treated rats following endotoxin and in rats pretreated with the PI derivative. These lesions were consistently not detected in rats pretreated with the PA isomer. In one series 4 h after endotoxin, gross observations revealed splanchnic infarction and haemorrhage

Table 3 Effect of the pyridine active (PA) and inactive (PI) derivative on *in vitro* synthesis of $iTxB_2$, and i6-keto-$PGF_{1\alpha}$ by peritoneal macrophages[a]

Group	*$iTxB_2$* (ng/ml)	*i6-keto-$PGF_{1\alpha}$* (ng/ml)
Control culture	11.1 ± 1.1	17.6 ± 1.2
PA (150 μM)	3.0 ± 0.2*	38.2 ± 6.8*
(500 μM)	0.5 ± 0.2*	31.7 ± 4.2*
PI (150 μM)	8.5 ± 0.8	15.3 ± 2.0
(500 μM)	7.7 ± 0.8	17.0 ± 5.0

[a]Macrophages were incubated *in vitro* with PA and PI for 5 h ($n = 3$–5).
*$P < 0.01$ compared to control culture.

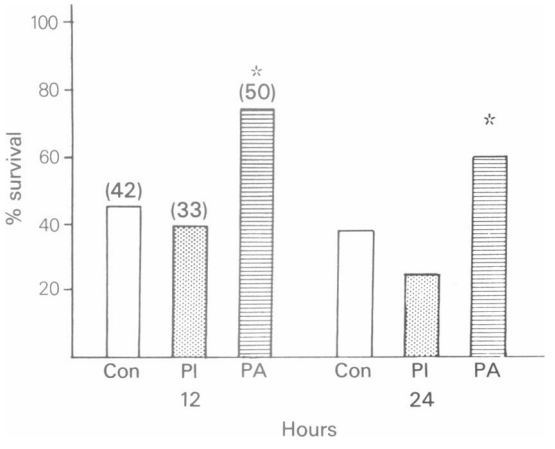

Figure 2 Mortality at 12 and 24 h after i.v. *S. enteritidis* endotoxin (12.5 mg/kg). PA =pyridine active thromboxane (Tx) synthetase inhibitor. PI = pyridine inactive Tx synthetase inhibitor. *n* in parentheses.
*$P < 0.05$ vs control rats.

were present in 4 out of 5 shocked controls, and 5 out of 5 rats pretreated with the PI compound, whereas none of the rats ($n = 5$) pretreated with PA compound showed any signs of infarction.

Lysosomal labilization, blood glucose, and hepatic dysfunction

Pretreatment with the PA but not the PI derivative significantly ($P < 0.05$) decreased serum β-glucuronidase compared to shocked vehicle-treated rats. Endotoxin-induced elevation in serum acid phosphatase levels were not decreased by the PA compound but were significantly ($P < 0.01$) increased by pretreatment with the PI compound relative to shocked controls (Table 4).

Pretreatment with the PA isomer, but not the PI isomer, prevented endotoxin-induced hypoglycaemia ($P < 0.01$) relative to shocked controls. Similarly plasma GOT and GPT were significantly reduced ($P < 0.05$) by the PA derivative compared to the other groups (Table 5).

Serum fibrin/fibrinogen degradation products (FDP)

Serum FDP were measured as an indication of the severity of disseminated intravascular coagulopathies. Rats pretreated with the PA derivative exhibited significantly ($P < 0.01$) lower increases in FDP compared to shocked control rats. Serum FDP levels in rats treated with the PI compound were not significantly different from the shocked control group (Table 6).

Discussion

This study has demonstrated that a pyridine derivative with an alkyl side chain substituted in the *meta* position (PA) is an effective inhibitor of endotoxin-stimulated TxB_2 synthesis both *in vivo* and *in vitro*. On the other hand, the *ortho* isomer (PI) did not prevent endotoxin-induced increases in plasma $iTxB_2$ *in vivo*. Furthermore, *in vitro* incubation of peritoneal macrophages with the PA derivative (150 μM) produced a 73% inhibition of $iTxB_2$ synthesis while its *ortho* isomer (500 μM) had no significant effect. This structure-activity relationship is similar to that reported by Tai, Lee & Tai, 1980. The inactive Tx synthetase inhibitor, thus provided a control for other potential salutary actions of the active isomer in endotoxic shock.

This study confirms and extends previous observations that Tx synthetase inhibitors may be beneficial

Table 4 Effect of the pyridine active (PA) and inactive (PI) derivative on endotoxin (LPS) induced lysosomal labilization[a]

Group	*β-Glucuronidase*[b] (units/ml)	*Acid phosphatase*[c] (units/ml)
Vehicle + LPS	410 ± 82 (16)	4.9 ± 0.4 (16)
PA + LPS	223 ± 18* (15)	4.3 ± 0.4 (15)
PI + LPS	685 ± 114 (5)	7.9 ± 1.2** (5)

[a]Studies conducted 4 h after *S. enteritidis* endotoxin. Compound PA and PI was administered i.p. (30 mg/kg) 30 min before LPS (15 mg/kg). *n* in parentheses.
[b]Normal values = 44 ± 14 units/ml ($n = 7$), Halushka *et al.*, 1981.
[c]Normal values = 1.6 ± 0.5 units/ml ($n = 7$), Halushka *et al.*, 1981.
*$P < 0.05$ compared to vehicle + LPS group.
**$P < 0.01$ compared to vehicle + LPS group.

Table 5 Effect of the pyridine active (PA) and inactive (PA) derivative on endotoxin (LPS)-induced hypoglycaemia and hepatic dysfunction[a]

Group	*Blood glucose* (mg%)	*Plasma GOT*[b] (SF/Units)	*Plasma GPT*[c] (SF/Units)
Vehicle + LPS	33.0 ± 5.0 (33)	392 ± 47 (16)	232 ± 50 (11)
PA + LPS	68.0 ± 5.3** (32)	273 ± 29* (15)	102 ± 17* (11)
PI + LPS	26.0 ± 21.4 (5)	679 ± 144* (5)	395 ± 101 (5)

[a]Studies were conducted 4 h after *S. enteritidis* endotoxin. Pyridine compounds were administered i.p. (30 mg/kg) 30 min before endotoxin. *n* in parentheses.
[b]Normal values = 72 ± 7 SF units/ml ($n = 7$), Cook *et al.*, 1981b.
[c]Normal values = 18 ± 5 SF units/ml ($n = 7$), Cook *et al.*, 1981b.
*$P < 0.05$ compared to vehicle + LPS group.
**$P < 0.001$ compared to vehicle + LPS group.

in experimental endotoxic shock in the rat. The inhibition of endotoxin-induced $iTxB_2$ synthesis with the PA derivative was associated with significantly enhanced survival time, decreased lysosomal labilization, serum fibrin degradation products, hepatocellular dysfunction, and less severe hypoglycaemia. However, plasma $iTxB_2$ levels in rats pretreated with the PI compound did not differ from shocked controls, and there was no change in mortality or attenuation of shock sequelae.

The *meta* substituted pyridinylmethyl amino benzoic acid but not the *ortho* substituted isomer prevented splanchnic infarction similar to that seen with the Tx synthetase inhibitor UK 37,248 (Halushka *et al.*, 1982). LeDuc & Needleman (1979) have previously demonstrated that the intestinal tract is capable of synthesizing both $iTxB_2$ and i6-keto-$PGF_{1\alpha}$. Cook, Halushka, Wise & Tempel (1982) have found that intravenous endotoxin induced a significant increase in portal venous TxB_2 compared to arterial TxB_2 in the rat. Thus the reduction in splanchnic infarction may be a result of inhibition of thromboxane synthesis within the intestinal mucosal and serosal layers with a resulting decrease in vasomotor tone and/or microthrombi formation. Furthermore the thromboxane synthetase inhibitors, 7-IHA and UK37,248 and OKY 1581 (a pyridine derivative) significantly improved endotoxin-induced decreases in splanchnic blood flow in the rat (Tempel, Cook, Wise & Halushka 1982).

As anticipated the Tx synthetase inhibitor, PA, did not significantly inhibit the early 30 min induced increase in i6-keto-$PGF_{1\alpha}$, but unexpectedly it significantly reduced the increase at 4 h. This compound does not appear to inhibit fatty acid cyclo-oxygenase since it did not significantly depress endotoxin-induced increases in PGE. Furthermore, even at 500 μM concentrations, the derivative, caused shunting *in vitro* to i6-keto-$PGF_{1\alpha}$ synthesis by peritoneal macrophages. The fact that i6-keto-$PGF_{1\alpha}$ levels were significantly decreased in rats pretreated with the PA derivative, may reflect a less severe shock

Table 6 Effect of the pyridine active (PA) and inactive (PI) derivatives on endotoxin (LPS)-induced elevations in fibrin/Fibrinogen degradation products (FDP)[a]

Group	*FDP* (μg/ml)
Vehicle + LPS	31.5 ± 7.2 (21)
PA + LPS	9.8 ± 1.6* (26)
PI + LPS	15.3 ± 3.4 (21)

[a]Measurements were made 4 h after *S. enteritidis* endotoxin. Compound PA and PI were administered i.p. (30 mg/kg) 30 min before endotoxin. *n* in parentheses.
Vehicle only values are < 0.5 μg/ml.
*$P < 0.01$ compared to the vehicle group.

state which in turn may have resulted in a lesser stimulus for the synthesis of PGI_2. Pharmacological infusions of PGI_2 during early endotoxic shock have been shown to be protective possibly via its vasodilating or platelet antiaggregatory properties (Fletcher & Ramwell, 1980b; Krausz, Utsunomiya, Feuerstein, Wolfe, Shepro & Hechtman, 1981). Our observations do not support the notion that the protective action of Tx synthetase inhibitors are necessarily mediated by *in vivo* shunting to PGI_2. It may be argued that the increased PGI_2 formed may act on vascular smooth muscle and platelets unopposed by TxA_2 when synthesis of the latter is depressed. While this notion is tenable and difficult to test directly, it must be considered. However, synthesis of PGI_2 is not required to maximize survival since non-steroidol anti-inflamatory drugs which completely inhibit the metabolism of arachidonic acid enhance survival and/ or prevent certain sequelae of endotoxaemia, (Parratt & Sturgess, 1975; Fletcher & Ramwell, 1980a; Wise *et al.*, 1980b; Halushka, Wise & Cook, 1981). Additionally essential fatty acid deficiency which improves survival against endotoxic shock or survival time in septic shock is associated with reduced arachidonic acid synthesis (Cook, Wise, Knapp & Halushka, 1981b; Butler, Wise, Halushka & Cook, 1981). We have observed marked increases in endogenous PGI_2 synthesis in the delayed phase of endotoxic shock (Cook *et al.*, 1981; Halushka *et al.*, 1982) and septic shock in the rat (Butler *et al.*, 1981). Similar observations have been made during endotoxic shock in the rabbit (Bult, Beetens & Herman, 1980) and baboon (Harris *et al.*, 1980). This suggests that PGI_2 may therefore mediate counter productive vasodilatation and contribute to systemic hypotension.

In summary, a pyridine-based Tx synthetase inhibitor, has beneficial effects in endotoxin shock in the rat. This beneficial effect appears to be mediated by inhibition of TxA_2 synthesis since pretreatment with an inactive *ortho* isomer did not attenuate shock sequelae in this model of endotoxic shock.

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