Degradation of prostaglandin F_2a in the human pulmonary circulation

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Jose, P., Niederhauser, U., Piper, Priscilla J., Robinson, Christine, and Smith, A. P. (1976). *Thorax*, **31**, 713–719. **Degradation of prostaglandin $F_2\alpha$ in the human pulmonary circulation.** Degradation of prostaglandins (PGs) during passage through the human pulmonary circulation was investigated by measuring the transpulmonary plasma $PGF_2\alpha$ difference during continuous intravenous infusion of $PGF_2\alpha$ (5–10 μg/min). Seven patients with cardiological disorders and two patients with extensive pulmonary abnormalities were investigated during diagnostic cardiac catheterization. $PGF_2\alpha$ levels were measured by radioimmunoassay. The seven cardiac patients were found to have transpulmonary $PGF_2\alpha$ differences of 47–88%, indicating metabolism of the PG in the lungs. A patient with extensive bronchiectasis had an apparently normal transpulmonary $PGF_2\alpha$ difference despite gross abnormalities in routine lung function tests. A patient with primary pulmonary arterial hypertension showed no metabolism of $PGF_2\alpha$ in the pulmonary circulation. The results show that PG degradation is an aspect of normal lung function and suggest that it becomes impaired when extensive pulmonary vascular damage exists.

Mammalian lungs carry out important metabolic processes in addition to their respiratory functions (Heinemann and Fishman, 1969). These include release of biologically active mediators either from preformed stores, for example histamine, or synthesis *de novo* before release, for example, slow reacting substance of anaphylaxis (SRS-A) and prostaglandins (PGs). The passage of certain molecules through the pulmonary circulation results in their metabolism; this may either increase biological activity, as in the case of angiotensin I being converted to angiotensin II, or decrease activity, as in the case of kinins and PGs (for references, see Bakhle and Vane (1974)). When PGs of the E and F series pass through the pulmonary circulation of a number of animal species they are metabolized in such a way that their biological activity is decreased by 90–95% (Ferreira and Vane, 1967). When PGE_2 (5–10 μg/min) was infused intravenously in man it had slight or variable effects on forced expira-

tory volume in one second (FEV_1) (Smith, 1974). This may have been due to metabolism of PGE_2 in the human pulmonary circulation, as occurs in other animals (Piper, Vane, and Wyllie, 1970). The purpose of this investigation was to study the fate of $PGF_2\alpha$ infused into the human pulmonary circulation. We carried out investigations in patients undergoing cardiac catheterization who had either apparently normal pulmonary function or extensive pulmonary abnormalities. Part of this work has already been communicated to the British Pharmacological Society (Jose *et al.*, 1976).

METHODS

Nine patients aged between 18 and 67 agreed to participate in the experiment after its nature and purpose had been described. Seven, three of whom had noticed shortness of breath on exertion, had been admitted to hospital for diagnostic

cardiac catheterization; the remaining two patients, whose illnesses are summarized below, were in hospital for investigation and treatment of their lung conditions.

The experiment was performed during the cardiac catheterization procedure after the necessary pressure measurements had been taken, but before angiography. With the catheters *in situ*, 18 ml of blood was withdrawn into 2 ml of saline or Krebs solution containing heparin (1000 units) and indomethacin (200 µg). The final concentration of the indomethacin was therefore 10 µg/ml and the plasma was diluted by a factor of approximately 1·2. Venous blood was withdrawn from the pulmonary artery catheter and arterial blood from either the aorta, if a left heart catheter had been introduced, or from a cannula introduced under local anaesthesia into a peripheral artery.

$PGF_2\alpha$ (tromethamine salt, Prostin $F_2\alpha$, Upjohn Co., Kalamazoo) was then infused at 5–10 µg/min into a dorsal hand vein for a period of 10 min, during which pulmonary arterial pressure was continuously monitored, and further samples of mixed venous and arterial blood were obtained from the catheters.

The blood samples were kept on ice and centrifuged within 5 min in plastic tubes at 4°C for 15 min at 700 *g* (except where stated). In all but two cases (SP and EM) platelet-poor plasma was prepared by re-centrifuging the plasma at 1200 *g*. Triplicate 2 ml plasma aliquots (+100 µl internal standard, 400 dpm ≡ 3·6 pg ^3H-$PGF_2\alpha$) were acidified to pH 3·0–3·5 with 200 µl of N-hydrochloric acid and extracted twice with peroxide-free diethyl ether. The recovery of internal standard was usually 80–90%. The plasma concentrations of $PGF_2\alpha$ were measured by radioimmunoassay based on the method of Hennam *et al.* (1974). 100 µl aliquots of the following reagents in tricine buffered saline (pH 8·0+0·1% gelatin) were incubated at 2–6°C: prostaglandin (plasma extract or 3·9–500 pg of standard $PGF_2\alpha$), ^3H-$PGF_2\alpha$ (approx. 22 000 dpm ≡ 20 pg) and antiserum to $PGF_2\alpha$ (diluted 1 : 16 000 so that binding of tracer in the absence of added prostaglandin was 50–60%). The mixtures were incubated for 1–2 h although equilibrium was reached after 30 minutes. The protein-bound fraction was precipitated by ammonium sulphate followed by centrifugation at 1500 *g* for 10 minutes. The pellet was dissolved in 400 µl of H_2O. 1 ml of Instagel (Packard) was added and the radioactivity present in the resulting gel was counted in a Packard Tri-Carb Liquid Scintillation Counter using the

sample channels ratio method to calculate counting efficiency. The method blank was determined by assaying the ether extract of heparin and indomethacin in saline and was approximately 60 pg/ml, but the smallest amount of $PGF_2\alpha$ which could be reliably measured (that which displaced 15% of the tritiated $PGF_2\alpha$ from the antiserum) corresponded to a plasma concentration of 100 pg/ml. When samples of venous and arterial plasma collected during the $PGF_2\alpha$ infusions were compared over a range of dilutions with standard $PGF_2\alpha$, the dose–response curves were parallel. The cross-reactions of the antiserum with other PGs and metabolites are shown in Table I.

TABLE I
REACTIONS OF PROSTAGLANDINS AND METABOLITES WITH THE ANTI $PGF_2\alpha$ SERUM RELATIVE TO $PGF_2\alpha$

Prostaglandin	n	Cross-reaction (%)[1]
$F_2\alpha$	4	100
15-keto-$F_2\alpha$	3	1·7±0·2
13, 14-dihydro-15-keto-$F_2\alpha$	4	0·8±0·3
13, 14-dihydro-$F_2\alpha$	3	3·0±0·5
$F_1\alpha$	2	17·5
E_2	3	0·02

n=number of determinations.
24 000 dpm (22 pg) of ^3H-$PGF_2\alpha$ was added to all tubes. Binding in the absence of added PG was 52·4±7·4 (%, mean±SD).
[1]Calculated from the mass of PG required to displace 50% of the ^3H-$PGF_2\alpha$ from the antiserum.

Shortly after the catheterization procedure each patient attended the lung function laboratory for spirometry (using a dry bellows spirometer), single breath carbon monoxide transfer test (Ogilvie *et al.*, 1957), measurement of airways conductance, and subdivisions of lung volume (Dubois *et al.*, 1956a; Dubois, Botelho, and Comroe, 1956b), arterial blood, and alveolar gas analysis.

Of the two patients with lung disease, one (VB), a man aged 67, was shown to have extensive bilateral basal bronchiectasis; the other (DS), with primary pulmonary artery hypertension, is described below.

CASE REPORT

A 51-year-old Kosher butcher (DS) presented with severe exertional dyspnoea, which had become progressively worse since its onset three months previously after a short febrile illness. He had received no drugs other than a course of antibiotics. Physical examination revealed central

cyanosis and finger clubbing, elevation of the jugular venous pressure with marked 'a' waves, accentuation of the pulmonary second sound, and hepatomegaly.

Chest radiographs showed prominent pulmonary arteries and the electrocardiogram showed right bundle-branch block and right ventricular hypertrophy. The results of tests of pulmonary function are summarized in Table III. Bronchoscopy, leg venograms, a venacavagram, and right heart angiography were normal, but pulmonary arteriography revealed multiple small vascular occlusions (Figure). A diagnosis of primary pulmonary arterial hypertension was made and treatment was begun. Despite anticoagulants, dipyrimadole, and continuous oxygen for 18 hours a day, he remained severely disabled and died 14 months after the onset of the illness after a myocardial infarction. His relatives withheld permission for a necropsy.

RESULTS

The results are summarized in Tables II and III.

The mixed venous plasma $PGF_2\alpha$ concentration (mean$\pm$SEM) of the patients without lung disease who received 10 μg $PGF_2\alpha$/min was 2620 $\pm$540 pg/ml. The concentration of $PGF_2\alpha$ in the arterial plasma was 800$\pm$250 pg/ml, indicating a mean transpulmonary difference of 69% (range 47–83%). Patient NC, who received the infusion at a rate of 5 μg/min, had somewhat lower plasma concentrations but a similar or slightly higher transpulmonary PG difference (88%). The infusion of $PGF_2\alpha$ did not cause any change in pulmonary arterial or aortic pressures, nor did it have any detectable systemic effects.

The determined pre-infusion plasma $PGF_2\alpha$ concentrations ranged between 30 and 170 pg/ml (mean=93). The levels found in patients SP and EM were higher than in the other subjects, possibly because these plasma samples were not necessarily 'platelet-poor' and were centrifuged at room temperature (SP) or in glass tubes (EM), two conditions which are known to be capable of releasing PGs from human platelets (Silver *et al.*, 1972). The method blank (which was not
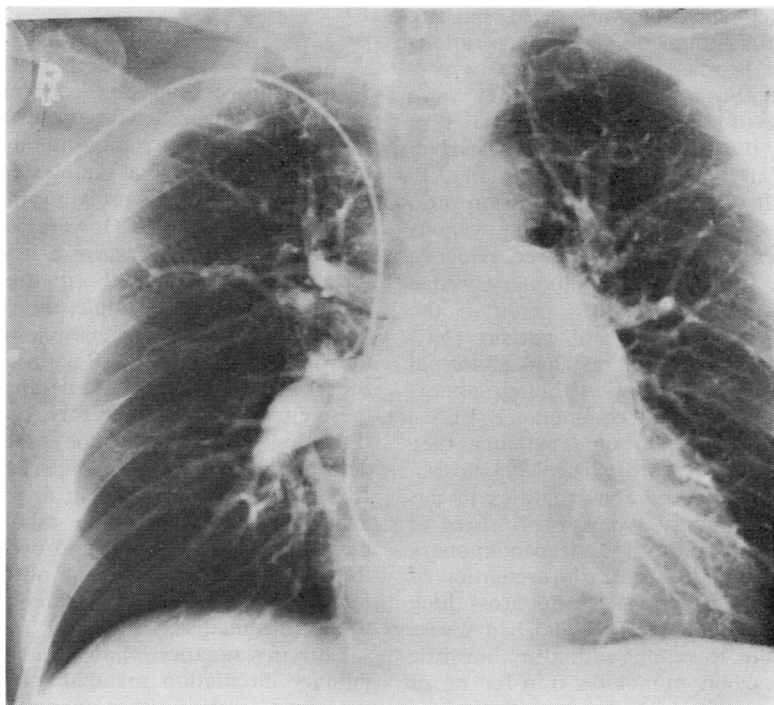

FIGURE *Pulmonary arteriogram of patient DS, showing extreme vascular abnormalities.*

TABLE II

PLASMA $PGF_2\alpha$ VALUES BEFORE AND DURING INFUSION OF $PGF_2\alpha$

Patient	$PGF_2\alpha$ Infusion (μg/min)	Plasma Concentration of $PGF_2\alpha$ (pg/ml)				% Transpulmonary Difference
		Pre-infusion[1]		Infusion		
		Venous	Arterial	Venous	Arterial	
SP[2]	10	170	140	1550	830	47
EM[3]	10	120	130	1830	460	75
PL[4]	10	40	30	2480	430	83
NC	5	90	70	1450	170	88
JC	10	30	30	1800	510	72
AP	10	90	150	5120	2000	61
LP	10	120	110	2930	550	81
VB	10	110	110	2980	900	70
DS	5	70	70	1670	1660	0·5

[1]Nominal values only (see text).
[2]Blood centrifuged at 20°.
[3]Blood centrifuged in glass tubes.
[4]Anticoagulant used was ethylene diamine tetra acetic acid (sodium salt).

subtracted from the calculated values) ranged from 30 to 80 pg/ml, and it was not possible to measure plasma $PGF_2\alpha$ concentrations of less than 100 pg/ml accurately. Therefore the pre-infusion values shown in Table II should be considered as nominal only. These values are higher than some previously published data for human peripheral venous plasma (Hennam et al., 1974; Dray, Charbonnel, and Maclouf, 1975), but are included for comparison with the concentrations found during the prostaglandin infusion.

Minor abnormalities in lung function were detected in some of these patients. Arterial Po_2, alveolar/arterial oxygen difference, and carbon monoxide transfer were reduced in patient AP, possibly the result of a ventilation/perfusion ratio disturbance caused by high pulmonary venous pressure. Another patient (EM) showed evidence of defective gas transfer, her pulmonary wedge and pulmonary arterial pressures being at the upper limits of normal; a third patient (NC) with normal gas exchange at rest had abnormal wedge and arterial pressures. Although abnormalities were found in airway conductance (FEV_1) and static lung volumes in some patients, they were consistent with the cardiological diagnosis.

Patient VB, with severe abnormalities in gas exchange, had pulmonary vascular pressures at the upper limits of normality and a transpulmonary $PGF_2\alpha$ difference of 70%, indicating retention of this aspect of lung function despite gross lung disease. By contrast, patient DS, who had severe arterial hypoxia due to venous admixture but little impairment of carbon monoxide transfer or of lung mechanics, was found to have similar mixed venous and arterial plasma $PGF_2\alpha$ levels, indicating failure of the degrading mechanism found in

the other patients. It was not possible confidently to exclude a right to left intracardiac shunt in this patient, but his calculated venous admixture of 39% was too small to account completely for the loss of pulmonary prostaglandin metabolism. His mixed venous PG values, however, were similar to those of the other patients, indicating that PG metabolism was probably occurring elsewhere.

DISCUSSION

The pre-infusion plasma concentrations of PGs were low compared with those found during infusion. Thus, the major immunoreactive substances in the plasma samples taken during the $PGF_2\alpha$ infusion were probably the parent $PGF_2\alpha$ and its metabolites. However, the pulmonary metabolites of $PGF_2\alpha$ have a low reactivity with the antiserum compared with that of the parent prostaglandin. For example, the most likely metabolite to appear in the plasma would be 13, 14-dihydro-15-keto $PGF_2\alpha$ (Beguin et al., 1972), and the cross-reaction of this metabolite is 0·8%. In these circumstances, therefore, radioimmunoassay (RIA) would appear to be a valid method for determining the plasma concentrations of $PGF_2\alpha$. In a preliminary experiment in which $PGF_2\alpha$ was infused intravenously in a rabbit, the transpulmonary difference was found by RIA to be 88%, that is, slightly less than the normal range determined by bioassay but greater than the values found in humans.

This investigation shows that the human pulmonary circulation metabolizes $PGF_2\alpha$ in much the same way as other mammalian lungs. The results show that of the $PGF_2\alpha$ infused into a peripheral vein only 30% survived passage

TABLE III
RESULTS OF LUNG FUNCTION TESTS AND CARDIOLOGICAL INVESTIGATIONS

Patient	Sex/Age	Diagnosis	PaO_2 (kPa)	$PaCO_2$ (kPa)	$A\text{-}aDO_2$ (kPa)	TCO (mmol min^{-1} kPa^{-1})	TLC (l)	VC (l)	$FEV_{1\cdot0}$ (l)	SGaw (s^{-1}kPa^{-1})	Ppa (mmHg)	Ppa(W) (mmHg)	$\dot{Q}$ (l/min)
SP	F 22	ASD	14·4	3·5	1·2	8 (8·7)	3·8(4·4)	2·6(3·0)	2·4(2·7)	1·8	25/10	5	—
EM	F 58	MS	12	4·4	3·3	4·3(7·0)	4·3(5)	1·8(3·2)	1·3(2·4)	2·0	30/15	15	—
PL	F 18	ASD	12·5	4·7	1·3	9·7(10)	—	3·8(3·8)	3·4(3·2)	—	23/7	7	—
NC	M 51	MI	12·8	4·0	1·5	7·0(9·7)	6·2(6·6)	4·5(4·4)	3·0(3·3)	1·2	50/26	24	—
JC	F 24	ASD	12·6	4·7	1·6	12·4(9)	4·7(5)	3·6(3·2)	2·3(2·8)	1·4	28/12	16	—
AP	M 53	AS	9·8	4·7	4·5	4·1(9·4)	4·5(5)	3·0(4·2)	2·2(3·1)	1·6	52/24	24	—
LP	M 54	MI	—	—	—	—	—	—	—	—	48/20	22	—
VB	M 67	Bronchiectasis	9·3	6·7	5·3	2·2(8·7)	—	2·5(3·8)	0·9(3·0)	—	33/12	12	5·3
DS	M 55	Pulmonary hypertension	6·1	4·6	5·2	8·4(9·7)	—	3·7(4·4)	2·7(3·3)	—	80/34	—	2·4

Predicted normal values in parentheses.
ASD = atrial septal defect.
AS = aortic stenosis.
MI = mitral incompetence.
MS = mitral stenosis.

through the lungs. PGE_1 is also degraded in human lungs by between 70 and 80% (Gillis, personal communication; Golub et al., 1975) although in these experiments PGE_1 was given either as bolus injections or hour-long infusions and estimated by methods other than radioimmunoassay. PGA_1 is metabolized to a lesser extent than PGE_1 in the human pulmonary circulation, as in the lungs of cat and dog (Horton and Jones, 1969; McGiff et al., 1969; Golub et al., 1975).

PGE_1 and $PGF_2\alpha$ do not seem to be metabolized so completely in human as in other mammalian lungs as most mammalian lungs convert 90–95% of infused E and F-type PGs (Vane, 1969). Since the pulmonary metabolites of PGE_2 and $F_2\alpha$ have intrinsic biological activity (Crutchley and Piper, 1975; 1976) and estimations of metabolism of the parent PGs were originally carried out by means of bioassay, the metabolism of PGs in mammalian lungs may be even higher than stated values. The difference between the extent of metabolism in human and other mamalian lungs may be a true species difference or may be explained by the physical conditions of the patients requiring cardiac catheterization or the fact that the patient had been sedated with valium whereas the animals were anaesthetized.

The exact site of metabolism of PGs in the pulmonary circulation is not known but other enzymes causing metabolism of biologically active substances have been found in the caveolae of the pulmonary endothelial cells (Smith and Ryan, 1973). If the enzymes for metabolism of PGs are located in a similar position, damage to the pulmonary endothelium would be expected to impair degradation of prostaglandins. This could explain our finding that the patient (DS) with an unidentified but probably primary abnormality of the pulmonary circulation was unable to metabolize exogenous $PGF_2\alpha$.

It is of interest that the patient VB with bronchiectasis, despite severe abnormalities in gas exchange and lung mechanics, showed no alteration of PG metabolism. As Piper et al. (1970) have already shown that considerable reserve capacity for PG degradation exists in the guinea-pig lung, extensive pulmonary damage is probably necessary before this process becomes impaired.

A possible function of PGs released in the lung may be local modulation of blood flow and ventilation. Alteration of synthesis and pulmonary metabolism of PGs in disease could lead to changes in ventilation/perfusion ratios. For example, it has been suggested that hypoxia in the lungs leads to PG synthesis (Said, Hara, and Yoshida, 1975), but that increased oxygen tension impairs metabolism of PGs (Parkes and Eling, 1975). Since patient DS had undergone oxygen therapy this may have contributed to the inability to metabolize $PGF_2\alpha$ in the pulmonary circulation.

It appears that metabolism of PGs is an aspect of normal human lung function. Extensive pulmonary vascular damage is probably necessary before it becomes impaired and the extent, and significance, of such impairment in lung disease remain to be discovered.

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