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Pulmonary hypertension in infants with congenital heart defects: are leukotrienes involved?

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The circulating levels of leukotriene E_4 in infants with congenital heart defects, increased pulmonary blood flow and pulmonary arterial hypertension, were determined and compared with infants with decreased pulmonary blood flow (Tetralogy of Fallot). There was no correlation ($r = 0{\cdot}38$) between the pulmonary arterial pressure (56 ± 4 mmHg) and the leukotriene E_4 levels ($1{\cdot}37 \pm 0{\cdot}67$ ng/ml blood) measured in peripheral blood samples from the hypertensive group prior to surgery. There was considerable variation in the detectable leukotriene E_4 levels in blood samples from different patients. The levels detected in the blood samples between the two groups of patients was similar. These data suggest that neither the surgical repair during cardiopulmonary bypass nor the pulmonary hypertension appeared to modify the leukotriene E_4 blood levels in the small number of patients studied.

Key words: Pulmonary hypertension, Cardiovascular surgery, Congenital heart defects, Leukotrienes, Extracorporeal circulation

Introduction

The hypothesis that metabolites of the arachidonic acid cascade, specifically products of the 5-lipoxygenase pathway, may be involved in pulmonary hypertension was based on two lines of evidence. First, injection of cysteinyl-leukotrienes significantly increased pulmonary vascular resistance in neonatal lambs[1] as well as in mature guinea-pigs and rats.[2] Furthermore, in the monkey, transient pulmonary hypertension was also observed following leukotriene administration.[3] The second line of evidence was provided by Stenmark and coworkers[4] who detected the presence of cysteinyl-leukotrienes in newborns with persistent pulmonary hypertension. These latter observations were based on analysis of bronchoalveolar lavage fluid derived from infants with pulmonary hypertension. Such data suggested indirectly that cysteinyl-leukotrienes which are potent vasoconstrictor agents in the human lung[5] may be associated with elevated pulmonary arterial pressure in children.

Previous surgical reports have shown that there are acute transitory episodes of increased pulmonary arterial pressure in patients following open heart surgery for correction of congenital heart lesions.[6] Unfortunately, the circulating levels of leukotrienes in this subset of patients is not known. In this study, the levels of LTE_4 in blood samples derived from infants with congenital heart defects, increased pulmonary blood flow and pulmonary hypertension were determined during the surgical intervention for correction of the heart defect and compared with infants with decreased pulmonary blood flow. Since LTE_4 is known to be produced by the human lung *in vitro*[7,8] and is a stable product of the 5-lipoxygenase pathway, the aim of the present study was to establish whether or not circulating LTE_4 was correlated with pulmonary arterial pressure and to determine whether the human lung *in situ* released this 5-lipoxygenase pathway metabolite.

Methods

Subjects

Eighteen patients with congenital heart defects (2–25 months of age) were studied. Six were diagnosed to have regular form of Tetralogy of Fallot, seven patients had complete atrioventricular septal defect, three had truncus arteriosus and two had ventricular septal defect. All patients with pulmonary hypertension ($n = 12$) presented with heart failure despite medical therapy support. None of them received medication that could interfere with the arachidonic acid cascade. Haemodynamic measurements

(pulmonary, systemic and atrial pressures) were continuously recorded for each patient using the Hewlett Packard 78354A monitor. Open heart repair was performed with the aid of hypothermic cardiopulmonary bypass. Aortic cross-clamping with injection of blood cardioplegia was used during the intracardiac repair. The mean duration of the cardiopulmonary bypass in Fallot patients was 69 ± 4 min and 114 ± 9 min in pulmonary hypertension subjects. None of the patients had cardiac repair under deep hypothermic circulatory arrest. After sternal and pericardial opening, blood samples were drawn from the main pulmonary artery and the left atrium. Surgery was then conducted as usual. Prior to weaning from cardiopulmonary bypass, monitoring lines (Seldicath 3 Fr. Plastimed) were introduced in the left atrial cavity and in the main pulmonary artery. Whilst in the intensive care unit, blood samples were collected from the patients via these catheter lines.

Leukotriene E_4 measurements

All blood samples (0.5 ml) were collected directly in tubes containing methanol at the beginning (Start) of extracorporeal circulation (ECC), 5 min after lung reperfusion (RP) and at the end of ECC. The tubes were stored overnight at −20°C. The samples were then thawed, vortexed and centrifuged (5000 rpm for 20 min at 4°C). The supernatant was then removed and added to tubes containing 40 ml of methanol at 10% and this mixture was then passed through a column (Sep-Pak C-18). The extracts containing lipids were collected on 3 ml of methanol. These samples were then separated into equal volume aliquots and dried using a Speed-Vac evaporator. The residue was dissolved in mobile phase solution of HPLC (acetonitrile/water/acetic acid at pH 5.6). This solution (20 µl) was injected into an HPLC (Waters) for separation. The samples were collected and quantification was performed by EIA (Stallergens, Fresnes, France).

Due to the complexity of the surgical intervention, internal standards for LTE_4 were not performed. However, using a blood sample obtained prior to surgery in one infant the analytic recoveries for standard LTE_4 were 78% for 0·5 ng/ml and 82% for 1 ng/ml, demonstrating that collection of blood samples in tubes containing methanol permitted adequate recoveries of LTE_4. The range of LTE_4 levels detected in the peripheral blood samples was 0 to 0·76 ng/ml (Fallot patients) and 0 to 7·2 ng/ml (pulmonary hypertension patients).

Statistical analysis

Results are expressed as means ± SEM. Since the number of patients studied was limited and the LTE_4 range was large, no statistical analysis was performed on the data. However, the data for each patient are presented.

Results

The pulmonary arterial pressure and the quantities of LTE_4 detected in peripheral blood samples derived from patients without (Fallot) and with pulmonary hypertension (PH) prior to surgery are shown in Table 1. There was no correlation between the elevated preoperative pulmonary arterial pressure and the levels of circulating LTE_4 detected in the peripheral blood samples ($r = 0{\cdot}38$). The LTE_4 levels detected in the infants during the course of the surgical intervention are presented in Tables 2 and 3 as well as in Fig. 1. The LTE_4 levels measured in blood samples derived from the pulmonary artery for the different patients (Fallot vs. PH) were similar. Values obtained in the samples from the left atrium in these two groups of patients were also similar. In five patients (PH) who were studied over the course of 12 h the LTE_4 levels were not altered when compared with those data obtained at the beginning of ECC.

In five of the 18 patients examined (one Fallot and four PH), the LTE_4 levels were below the threshold level of detection. Of the 13 patients where LTE_4 levels were detectable, eight subjects exhibited higher LTE_4 levels in blood samples derived from the left atrium than those levels detected in blood samples from the pulmonary artery during the lung reperfusion period. In two subjects, the LTE_4 levels in the left atrium were lower than those measured in the pulmonary artery blood samples. In two patients no samples were obtained and in another patient the values were the same. In addition, the total quantities of LTE_4 detected during the surgical intervention were: Fallot,

Table 1. Pulmonary arterial pressure and circulating leukotriene E_4 levels in infant patients

Patients	Age (months)	(*n*)	PAP (mmHg)	LTE_4 (ng/ml)
Fallot	11.9 ± 3.2	6	NM	0.44 ± 0.12
PH	8.5 ± 1.6	12	56 ± 4	1.37 ± 0.67

Values are means ± SEM from the number of patients studied (*n*). Age at time of surgery. PH = pulmonary hypertension; PAP = pulmonary arterial pressure (prior to surgery) and NM = not measured. The LTE_4 levels were determined in peripheral blood samples prior to surgery.

Table 2. LTE_4 levels (pg/ml) detected in blood samples obtained from infants (Fallot)

Patients	Age (months)	Peripheral blood	ECC					
			Start		Lung reperfusion		End	
			PA	LA	PA	LA	PA	LA
1	8	590	660	750	432	470	327	440
2	3.2	366	421	471	2010	1850	582	1880
3	15.8	700	1040	565	525	780	635	665
4	25.7	755	835	765	500	1105	1170	1320
5	10.4	0	0	0	0	0	0	0
6	8.5	246	224	632	NP	445	491	523

PA = pulmonary artery; LA = left atrium; 0 = below level of assay detection; NP = no sample; ECC = extracorporeal circulation; months = age at surgery.

Table 3. LTE_4 levels (pg/ml) detected in blood samples obtained from infants (PH)

Patients	Age (months)	PAP (mmHg)	Peripheral blood	ECC					
				Start		Lung reperfusion		End	
				PA	LA	PA	LA	PA	PA
1	13	83	406	188	746	149	340	138	253
2	9	60	630	312	1180	550	542	551	522
3	7.2	52	0	0	0	0	0	0	0
4	12	48	0	0	0	0	0	0	0
5	3.7	NM	155	209	603	NP	NP	161	681
6	15	70	570	1086	799	413	566	NP	NP
7	3.8	67	5090	6679	1610	7158	2317	2517	1389
8	4.8	NM	1217	244	1743	570	1832	206	1404
9	2.7	70	7172	5427	NP	532	6850	4350	5920
10	20.5	47	0	0	0	0	0	0	0
11	2.4	40	1170	1200	1310	801	1370	270	1500
12	7.8	50	0	0	0	0	0	0	0

PA = pulmonary artery; LA = left atrium; 0 = below level of assay detection; NP = no sample; ECC = extracorporeal circulation; NM = not measured; PAP = pulmonary arterial pressure (prior to surgery); months = age at surgery.

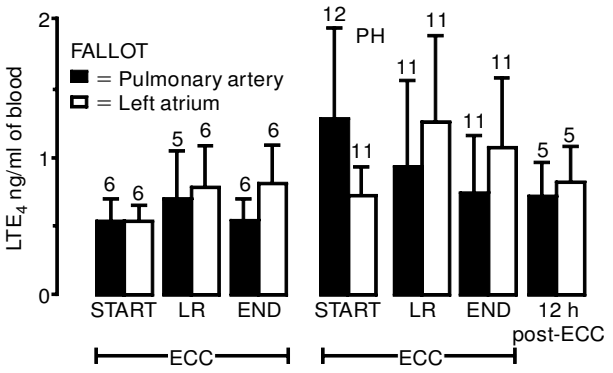

FIG. 1. Circulating leukotriene E_4 levels in infants during open heart surgery. Measurements of LTE_4 were performed in blood samples obtained during surgery in a group of infants without (Fallot) and with pulmonary hypertension (PH). Blood samples were derived from either the pulmonary artery or the left atrium at the beginning (START) of extracorporeal circulation (ECC), 5 min after lung reperfusion (LR) and at the END of ECC. In a limited number of subjects ($n = 5$) measurements were made at 12 h post-ECC. Values are means ± SEM and the number of infants studied is presented above each bar.

$0{\cdot}64 \pm 0{\cdot}17$ ng/ml blood; and PH, $1{\cdot}03 \pm 0{\cdot}43$ ng/ml blood.

Discussion

While LTE_4 was detected in blood samples from infants (72%) an increase in the circulating levels was not observed in those patients with pulmonary hypertension. These data, derived from a limited number of patients, suggested that neither the surgical intervention nor the pulmonary hypertension were associated with an alteration in LTE_4 blood levels.

There was considerable variation in the LTE_4 levels detected in blood samples obtained from neonates with or without pulmonary hypertension. The range in the quantities of LTE_4 detected was similar to those reported by other investigators when this metabolite was measured in either urine,[9–11] bronchoalveolar lavage[12] or blood samples[13] from patients with

different clinical pathologies. The reasons for these variations are presently unknown but are probably independent of the pathophysiological condition, since control subjects (Fallot) exhibit a similar range in LTE_4 values, when compared with values obtained in infants with pulmonary hypertension. Furthermore, the LTE_4 levels detected were not correlated with the high pulmonary arterial pressure observed in PH infants. These data are similar to a previous report in adult pulmonary hypertensive patients where other metabolites of the arachidonic acid cascade were measured.[14] These authors found no correlation between the pulmonary arterial pressure and thromboxane (TxB_2) levels in urine samples. These data suggest that the circulating potent vasoconstrictor metabolites of the arachidonic acid pathway (TxA_2 and LTE_4) which are detected in pulmonary hypertension may not be related to the *in vivo* pressure modulations reported in the pulmonary artery.

The low levels of LTE_4 (28% of patients, undetected) and the interpatient variation will now require further investigation. First, there may be a preferential metabolism of arachidonic acid in different individuals. A significant modification in the production/removal equilibrium of the 5-lipoxygenase metabolites could result in alterations in detection of the more stable metabolite (LTE_4). Therefore, the measurement of only LTE_4 in the biological samples may not be an appropriate index of the 5-lipoxygenase pathway activity in all patients. Unfortunately, no data are available concerning LTC_4 and LTD_4 levels in biological samples obtained from subjects where no LTE_4 was detected. The considerable variation in the levels of LTE_4 between patients may also be related to the way LTE_4 is bound in the circulation. Unfortunately, little is known about the fixation of cysteinyl-leukotrienes in the human circulation. Few studies have been published dealing with the quantitation of leukotrienes in human blood samples and analysis has been essentially based on results derived from human plasma.[15] Finally, an increase in quantities of leukotrienes may occur in the tissue compartment rather than in the circulation. Indeed, previous data have shown that following an instillation of LT in the rat lung only a small percentage could be detected in the lavage fluid[16] suggesting that the lung tissue rather than the lung liquid was the dominant compartment. Whether or not lung tissue from pulmonary hypertensive patients exhibits elevated levels of LTE_4 has not been reported. In order to adequately understand the role of these potent inflammatory mediators in pulmonary hypertension these issues will have to be addressed not only in relation to this disease but also other pathologies where leukotrienes have been implicated.

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