SHORT COMMUNICATION

Phospholipid Composition of Myocardium in Children with Normoxemic and Hypoxemic Congenital Heart Diseases

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Summary

Samples of myocardial tissue were obtained during cardiac surgery from children operated for different types of normoxemic and hypoxemic congenital heart diseases. The phospholipid composition was analyzed by thin layer chromatography. The concentration of total phospholipids (PL), phosphatidylcholine and phosphatidylethanolamine (PE) was found lower in atrial tissue of both normoxemic and hypoxemic groups in comparison with the ventricles. When comparing the difference between hypoxemic and normoxemic defects, hypoxemia was found to increase the concentration of total PL, PE and phosphatidylserine in ventricles and total PL and PE in the atria. The increased level of particular phospholipid species may represent adaptive mechanisms to hypoxemia in children with congenital heart diseases.

Introduction

      Congenital heart diseases are caused by abnormalities developed in the first six to eight weeks of fetal life. The incidence of heart malformations is about eight per thousand live births. These congenital cardiac defects may be grouped according to both the status of blood flow to the lungs and the presence and type of cardiac shunts. Right to left sided shunt mixes unoxygenated and oxygenated blood; oxygen blood saturation is thus significantly reduced and leads to hypoxemic (cyanotic) disease. On the other hand, left to right shunt is characteristic for normoxemic (acyanotic) disease. Recent medical progress in pediatric cardiac surgery allows successful repairing of almost all congenital heart defects. Nevertheless, only limited data are available on biochemical remodeling of both atrial and ventricular parts of diseased myocardium. We demonstrated that protein profiles of atrial musculature are totally different as compared with ventricular ones; the concentration of contractile proteins was higher in ventricle, while the concentration of extracellular matrix

proteins was higher in atrial musculature. Hypoxemia did not affect this protein profile in either cardiac part (Pelouch *et al.* 1993, 1995a, 1995b, 1997). There was no atrio-ventricular difference in the concentration of metabolic proteins but higher activity of carbohydrate and lipid aerobic metabolism enzymes was observed in ventricles of both normoxemic and hypoxemic patients (Bass *et al.* 1988, Pelouch *et al.* 1993). All metabolic differences mentioned above depended neither on the type of congenital heart disease nor on the reference values used (wet weight, metabolic or non-collagenous proteins) (Bass *et al.* 1988). The remodeling of human

cardiac phospholipids associated with aging and coronary heart disease was observed (Gudbjarnason 1989, Skuladottir *et al.* 1988). However, there are no available data dealing with phosholipid composition and their remodeling in human myocardium with congenital heart diseases. Therefore, the aim of this work was to determine phospholipid composition of atrial and ventricular musculature and to compare the effect of chronic normoxemic and hypoxemic defects on membrane phospholipid remodeling in children myocardium.

Table 1. Characterization of patients with congenital heart diseases

	normoxemic		hypoxemic	
	ventricle	atrium	ventricle	atrium
Age	10.0 ± 2.2	8.5 ± 2.5	4.8 ± 2.0	4.3 ± 1.2
pO_2 (%)	94.7 ± 1.2	95.0 ± 0.9	76.7 ± 2.0 *	78.1 ± 1.6 *

Values are mean ± S.E.M. *p<0.05, significant difference vs. normoxemic tissue.

Table 2. Phospholipid concentration (μmol P. g^{-1} w.w.) in myocardium from children with congenital heart diseases

Phospholipids	normoxemic		hypoxemic	
	ventricle	atrium	ventricle	atrium
PC	5.74 ± 0.39	3.07 ± 0.50 [#]	7.27 ± 0.91	4.44 ± 0.52 [#]
LPC	0.87 ± 0.20	n.d.	0.50 ± 0.16	0.58 ± 0.06
PE	3.96 ± 0.49	1.84 ± 0.40 [#]	5.80 ± 0.56 *	3.65 ± 0.48 [#] *
LPE	0.39 ± 0.10	0.27 ± 0.05	0.55 ± 0.10	0.47 ± 0.08
DPG	1.37 ± 0.14	0.78 ± 0.14	1.77 ± 0.30	1.28 ± 0.31
SM	1.04 ± 0.12	0.79 ± 0.13	0.94 ± 0.07	0.86 ± 0.09
PI	0.96 ± 0.10	0.52 ± 0.08	0.95 ± 0.20	0.73 ± 0.10
PS	0.38 ± 0.02	0.36 ± 0.03	0.53 ± 0.04 *	0.41 ± 0.04 [#]
Total PL	14.71 ± 0.81	8.01 ± 0.97 [#]	18.46 ± 1.42 *	12.42 ± 1.37 [#] *

Values are means ± S.E.M. PL (phospholipids), PC (choline phosphoglycerides), LPC (lysophosphatidylcholine), PE (ethanolamine phosphoglycerides), LPE (lysophosphatidylethanolamine), DPG (diphosphatidylglycerol), SM (sphingomyelin), PI (phosphatidylinositol), PS (phosphatidylserine). [#] p<0.05, significant difference atrium vs. ventricle; * p<0.05, significant difference hypoxemic vs. normoxemic tissue.

The study was performed on 23 cardiac tissue samples obtained during surgery of children (1 - 18 years) with congenital heart disease. The samples were taken from right atria (n=6) and right ventricles (n=6) of patients with normoxemic defects (ventricular or atrial septal defect), and also from right atria (n=5) and right ventricles (n=6) of patients with hypoxemic defects (tetralogy of Fallot). For further characterization of

patients see Table 1. The samples of cardiac tissue were rapidly weighed, frozen to –80 °C and stored at this temperature until the phospholipid analysis. The frozen tissue of both cardiac parts was pulverized and homogenized. Lipids were extracted according to the method of Folch *et al.* (1959) and evaporated under nitrogen. Phosphatidylcholine (PC), phosphatidyl-ethanolamine (PE), diphosphatidylglycerol (DPG),

sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylserine (PS) were separated by two-dimensional thin layer chromatography and the spots of individual phospholipids were analyzed for phosphorus (Rouser *et al*. 1970). Experimental values are given as means ± S.E.M. Differences were evaluated by one-way ANOVA and considered significant for p<0.05.

Table 1 confirms that the oxygen blood saturation was significantly lower in hypoxemic children than in normoxemic ones. As for the patients' age, we did not observe any significant difference either between atrial and ventricular defects or between normoxemic and hypoxemic ones. Table 2 shows that the concentration of total phospholipids (PL), PC and PE was significantly lower in atrial samples of both normoxemic (by 42 %, 47 % and 54 %, respectively) and hypoxemic groups (by

33 %, 39 % and 37 %, respectively) in comparison with the ventricles. Besides, we observed decreased concentration of PS (by 23 %) in hypoxemic atria in comparison with hypoxemic ventricles. Hypoxemia has a tendency to elevate the concentration of both total PL and individual phospholipid species. However, this increase was significant only in total PL (by 25 %), PE (by 46 %) and PS (by 39 %) in ventricular tissue and in total PL (by 55 %) and PE (by 98 %) in atrial samples. The relative proportion of individual phospholipids was unaltered with exception of the higher proportion of PS in normoxemic atria in comparison with normoxemic ventricles (by 83 %) and lower proportion of PS in hypoxemic atria in comparison with normoxemic ones (Table 3).

Table 3. Phospholipid distribution (%) in myocardium from children with congenital heart diseases

Phospholipids	normoxemic		hypoxemic	
	ventricle	**atrium**	**ventricle**	**atrium**
PC	39.00 ± 1.20	37.50 ± 2.50	38.80 ± 1.90	35.70 ± 0.90
LPC	6.18 ± 1.44	n.d.	3.93 ± 1.31	4.97 ± 0.82
PE	26.50 ± 2.20	26.10 ± 3.10	31.50 ± 1.40	29.10 ± 1.30
LPE	2.75 ± 0.76	3.87 ± 1.09	3.16 ± 0.58	3.89 ± 0.60
DPG	9.16 ± 0.56	9.84 ± 0.98	9.34 ± 0.89	9.82 ± 1.55
SM	7.22 ± 1.00	11.31 ± 2.63	5.23 ± 0.49	7.25 ± 1.16
PI	6.50 ± 0.44	6.45 ± 0.35	5.11 ± 0.50	5.90 ± 0.42
PS	2.69 ± 0.35	4.92 ± 0.75 [#]	2.93 ± 0.31	3.41 ± 0.33 *

For symbols see Table 2.

Our results demonstrate that the concentration of total PL is significantly higher in ventricles as compared with atria. This is due to the higher concentration of major phospholipids PC and PE, which reflects the higher content of intracellular membranes in ventricles. As the ratio of major phospholipids to mitochondrial DPG is similar in ventricles and atria, the higher total phospholipid concentration observed in ventricles is supposed to reflect the higher content of mitochondrial membranes. Moreover, higher activities of aerobic enzymes were found in ventricles in comparison with atria (Bass *et al.* 1988, Pelouch *et al*. 1988). The concentration of PL that we found in children heart was higher in comparison with values reported in adult human heart (Skuladottir *et al*. 1988). Although no data are available on developmental changes in phospholipid

concentration in human myocardium, it is known that during ontogenesis of rat myocardium phospholipid concentration gradually rises from the birth to the adulthood (Nováková *et al*. 1995). We did not observe any ontogenetic difference in our set of patients. The relative distribution of individual phospholipids determined in our study is similar to those found in other studies (Sebedio *et al*. 1982, Rocquelin *et al*. 1989). However, on contrary to these, we found the significant amount of lysophosphatidylcholine and lysophosphatidyl-ethanolamine in nearly all heart tissues under study. Because cardiac tissue of healthy children was not analyzed, we cannot claim that the presence of lysophospholipids was a consequence of heart disease; lysophospholipids might also originate from the process of tissue sampling during the surgery. We observed

higher concentration of aminophospholipids (PE, PS) in both atrial and ventricular tissues from children with hypoxemic defects as compared with normoxemic ones. We do not have a precise explanation for this phenomenon, but it is known that the redistribution of these phospholipid species in cardiac plasma membrane precedes the apoptotic cell death (Maulik *et al.* 1998). It is tempting to speculate that the changes in phospholipid composition observed in hypoxemic heart tissues may reflect either an adaptive response of the heart to the lower oxygen blood saturation or a sign of tissue damage.

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References

BASS A, ŠAMÁNEK M, OŠŤÁDAL B, HUČÍN B, STEJSKALOVÁ M, PELOUCH V: Differences between atrial and ventricular energy supplying enzymes in children. *J Appl Cardiol* **3**: 397-405, 1988.

FOLCH J, LEES M, SLOAN-STANLEY GH: A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* **226**: 497-509, 1957.

GUDBJARNASON S: Dynamics of n-3 and n-6 fatty acids in phospholipids of heart muscle. *J Intern Med* **225**: 117-128, 1989.

MAULIK N, KAGAN VE, TYURIN VA, DAS DK: Redistribution of phosphatidylethanolamine and phosphatidylserine precedes reperfusion-induced apoptosis. *Am J Physiol* **274**: H242-H248, 1998.

NOVÁKOVÁ O, MRNKA L, PELOUCH V, KALOUS M, NOVÁK F: Altered phospholipid composition and oxidative capacity in pressure-overloaded rat myocardium. *J Mol Cell Cardiol* **27**: A85, 1995.

PELOUCH V, MILEROVÁ M, OŠŤÁDAL B, ŠAMÁNEK M, HUČÍN B: Protein profiling of human atrial and ventricular musculature: the effect of normoxaemia and hypoxaemia in congenital heart diseases. *Physiol Res* **42**: 235-242, 1993.

PELOUCH V, MILEROVÁ M, OŠŤÁDAL B, ŠAMÁNEK M, HUČÍN B: Collagenous and noncollagenous proteins of human atrial and ventricular musculature: the effect of normoxaemia and hypoxaemia in congenital heart disease. *J Muscle Res Cell Motil* **16**: 171-172, 1995a.

PELOUCH V, MILEROVÁ M, OŠŤÁDAL B, HUČÍN B, ŠAMÁNEK M: Differences between atrial and ventricular protein profiling in children with congenital heart disease. *Mol Cell Biochem* **147**: 43-49, 1995b.

PELOUCH V, PEXIEDER T, MILEROVÁ M, OŠŤÁDAL B, ŠAMÁNEK M: Protein remodeling of malformed human and mouse heart. In: *The Developing Heart.* B OŠŤÁDAL, M NAGANO, N TAKEDA, NS DHALLA (eds), Raven Press, New York, 1997, pp 357-372.

ROCQUELIN G, GUENOT L, ASTORG PO, DAVID M: Phospholipid content and fatty acid composition of human heart. *Lipids* **24**: 775-780, 1989.

ROUSER G, FLEISCHER S, YAMAMOTO A: Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* **5**: 494-496, 1970.

SEBEDIO JL, FARQUHARSON TE, ACKMAN RG: Improved methods for the isolation and study of the C-18, C-20 and C-22 monoethylenic fatty acid isomers of biological samples-HG adducts, HPLC, AgNO$_3$-TLC/FID and ozonolysis. *Lipids* **17**: 469-475, 1982.

SKULADOTTIR G, BENEDIKTSDOTTIR E, HARDARSON T, HALLGRIMSSON J, ODDSSON G, SIGFUSSON N, GUDBJARNASON S: Arachidonic acid level of non-esterified fatty acid and phospholipids in serum and heart muscle of patients with fatal myocardial infarction. *Acta Med Scand* **223**: 233-238, 1988.

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