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Pulmonary arterial hypertension and type-I glycogen-storage disease: the serotonin hypothesis

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Pulmonary arterial hypertension and type-I glycogen-storage disease: the serotonin hypothesis. M. Humbert, P. Labrune, O. Sitbon, C. Le Gall, J. Callebert, P. Hervé, D. Samuel, R. Machado, R. Trembath, L. Drouet, J-M. Launay, G. Simonneau. ©ERS Journals Ltd 2002.

ABSTRACT: A case of pulmonary arterial hypertension in a patient with type-Ia glycogen-storage disease, a rare autosomal recessive disorder caused by a deficiency of glucose-6-phosphatase is reported in this study. It has been suggested that the occurrence of pulmonary arterial hypertension in type-Ia glycogen-storage disease could be due to an abnormal production of vasoconstrictive amines such as serotonin.

To test this hypothesis, plasma serotonin concentrations were prospectively measured in 13 patients with type-Ia glycogen-storage disease, one patient with severe pulmonary hypertension and type-Ia glycogen-storage disease, 16 patients displaying severe pulmonary arterial hypertension, and 26 normal healthy controls.

Elevated plasma serotonin concentrations were found in patients with either severe pulmonary arterial hypertension (38.8±7.3 nmol·L^{-1}) or type-Ia glycogen-storage disease (36.8±11.5 nmol·L^{-1}), as compared with controls (8.8±0.6 nmol·L^{-1}, $p<0.001$). Plasma serotonin was dramatically elevated in the patient with type-Ia glycogen-storage disease and pulmonary arterial hypertension (113.4 nmol·L^{-1}).

It is concluded that type-Ia glycogen-storage disease may be another condition in which abnormal handling of serotonin is one event in a multistep process leading to severe pulmonary arterial hypertension.

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Pulmonary arterial hypertension is characterised by the presence of pulmonary hypertension (mean pulmonary artery pressure >3.33 kPa (>25 mmHg) at rest or >3.99 kPa (>30 mmHg) during exercise) and normal pulmonary wedge pressure [1]. In the absence of any factor or condition suspected of playing a causal or facilitating role in the process, pulmonary arterial hypertension is unexplained (primary pulmonary hypertension) [2]. Type-I glycogen-storage disease is an autosomal recessive disorder caused by a deficiency of glucose-6-phosphatase (type-Ia, von Gierke's disease) or glucose-6-phosphate translocase (type Ib) [3]. Its estimated incidence is 1·100,000^{-1} [4]. Since the pioneering description by Pizzo [5] in 1980, six cases of severe pulmonary hypertension have been described in patients with type-Ia glycogen-storage disease [6–10]. In all cases this condition was very severe leading to the patients' death within a few months of cardiopulmonary arrest and/or untractable congestive right heart failure [5–10]. When available, *post mortem* examination of the lungs demonstrated pulmonary hypertensive arteriopathy, as described previously in primary pulmonary hypertension [5–8].

Pulmonary arterial hypertension, occurring in patients with rare diseases, provides valuable information for evaluating possible pathobiological hypotheses in pulmonary vascular disorders [2]. For instance, development of severe pulmonary arterial hypertension without any identifiable cause in a patient with a rare familial platelet storage deficiency allowed the authors to hypothesise that serotonin, a pulmonary vasoconstrictor and growth factor for vascular smooth-muscle cells stored in platelets, could play a role in pulmonary arterial hypertension [11–14]. In the initial report by Pizzo [5], it was suggested that the occurrence of pulmonary arterial hypertension in

type-I glycogen-storage disease could be due to an abnormal production of vasoconstrictive amines such as serotonin. As a first step in studying handling of serotonin in this rare condition, plasma serotonin concentrations in patients with type-Ia glycogen-storage disease were prospectively measured.

Patients and methods

In September 1997, a patient with type-Ia glycogen-storage disease was referred to the authors' hospital (Hôpital Antoire Béclère) because of severe pulmonary arterial hypertension. Three groups of patients were subsequently analysed in a prospective study designed to measure plasma serotonin in severe pulmonary arterial hypertension, type-Ia glycogen-storage disease without pulmonary hypertension, and normal healthy controls. All patients and controls were tested after appropriate consent was gained in accordance with the declaration of Helsinki.

Patient with type-Ia glycogen-storage disease and severe pulmonary arterial hypertension

A 25-yr-old male was referred to the authors' centre so that severe pulmonary hypertension could be investigated. As a child, the patient demonstrated growth retardation, hepatomegaly and fasting hypoglycaemia. A diagnosis of type-Ia glycogen-storage disease was established on a liver biopsy (hepatic glucose-6-phosphatase deficiency). Despite dietary treatment, the patient's condition did not improve significantly. At the age of 8 yrs a porto-caval shunt was performed as indicated at that time to try to improve the growth status. After surgery, growth was satisfactory (adult height: 1.73 m), and clinical stability was maintained for several years. At the age of 25 yrs the patient complained of dyspnoea on exertion (functional class II of the classification of the New York Heart Association). Physical examination showed marked smooth hepatomegaly and a loud pulmonic component of the second heart sound. Chest radiography revealed cardiomegaly and a convex main pulmonary artery. An electrocardiogram demonstrated a pattern of right ventricular hypertrophy. Echocardiography showed evidence of right ventricular hypertrophy and dilatation with an elevated systolic pulmonary artery pressure estimated at 10.64 kPa (80 mmHg). There was no evidence of congenital heart disease. Pulmonary embolism was excluded with a negative perfusion scan. Lung function was within the normal range. Right-heart catheterisation confirmed severe pulmonary arterial hypertension (table 1). The patient could walk 450 m in 6 min with a mild desaturation on exercise (decreasing from 98% to 94%). Echography and computed tomodensitometry of the liver demonstrated a patent porto-caval shunt and multiple adenomas of the liver. The patient was treated with conventional therapy, including exercise limitation, warfarin at sufficient doses to increase the international normalised ratio to ~2.0, and diuretics. The patient has subsequently been followed-up in the authors' centre without significant modifications of the clinical and haemodynamical status (table 1).

Patients with type-Ia glycogen-storage disease and no pulmonary hypertension

Of the 30 patients with type-Ia glycogen-storage disease regularly followed-up between 1997–1999, 13 (12±10-yr-olds) were randomly analysed in this study. Pulmonary hypertension was ruled out by clinical examination and a normal transthoracic echography with Doppler.

Patients with severe pulmonary arterial hypertension in the absence of glycogen-storage disease type Ia

During the same period (1997–1999), 16 patients referred to the hospital for initiation of continuous intravenous epoprostenol therapy were included in the present study (six with pulmonary arterial hypertension associated with a past history of appetite

Table 1. – A 4-yr follow-up of a patient with severe pulmonary hypertension and type-Ia glycogen-storage disease

	Year			
	1996	1997	1998	1999
Right atrial pressure kPa (mmHg)	ND	0.93 (7)	1.86 (14)	1.73 (13)
Systolic pulmonary artery pressure kPa (mmHg)	80[¶]	13.3 (100)	15.69 (118)	11.97 (90)
Mean pulmonary artery pressure kPa (mmHg)	ND	8.65 (65)	10.50 (79)	8.38 (63)
Mean pulmonary capillary wedge pressure kPa (mmHg)	ND	1.20 (9)	1.20 (9)	0.93 (7)
Cardiac index $L \cdot min^{-1} \cdot m^{-2}$	ND	3.30	3.77	3.32
Pulmonary vascular resistance kPa (mmHg) $L \cdot min \cdot m^{-2}$	ND	2.06 (15.5)	2.77 (20.8)	2.53 (19.0)
Mixed venous oxygen saturation %	ND	59	58	59
Acute vasodilator response[#]	ND	Negative	Negative	ND
6-min walk test m	ND	450	510	450
NYHA functional class	II	II	II	II
Pulmonary hypertension therapy	None	Diuretics+ warfarin	Diuretics+ warfarin	Diuretics+ warfarin

ND: not determined; NYHA: New York Heart Association. [#]: response to nitric oxide 10 ppm; [¶]: systolic pressure measured echography with doppler.

Table 2. – Patients with primary pulmonary hypertension

Age yrs	42±3
Right atrial pressure kPa	1.60±0.53
Mean pulmonary artery pressure	9.18±0.53
Cardiac index L·min·m^{-2}	2.11±0.13
Pulmonary vascular resistance kPa L·min·m^{-2}	4.70±0.52
Mixed venous oxygen saturation %	57±9
Acute vasodilator response#	Negative
6-min walk test m	323±38

Data are presented as mean±SEM. #: response to nitric oxide, 10 ppm.

suppressant exposure, nine with sporadic and one with familial primary pulmonary hypertension). Clinical and haemodynamical information, before initiation of epoprostenol therapy, are listed in table 2. Plasma serotonin was measured in all patients before epoprostenol therapy. In patients with a history of fenfluramine intake, fenfluramines exposure was stopped several years before analysis.

Controls

Twenty-six normal, age-matched healthy controls were included in parallel, corresponding to 10 individuals aged <18 yrs and 16 adults (>18 yrs).

Methods

In all patients and controls, 4.5 mL of blood was drawn by venipuncture from a peripheral vein in 0.5 mL of 0.129 M trisodium citrate. Platelet-rich plasma was prepared by centrifugation of citrated blood at 120×*g* for 10 min. Platelets were counted and platelet-rich plasma was further centrifuged at 2,000×*g* for 10 min in order to harvest separately the platelet pellets and the platelet-poor plasma. Serotonin levels were measured by radioenzymology and high-performance liquid chromatography in citrated whole blood, platelet-poor plasma (also referred to as plasma) and platelet pellet, as described previously [15].

Statistical analysis

Data were analysed with a Macintosh computer (Apple Company, New York, NY, USA) using the Stalview® 4.5 Software (Abacus Concepts Inc., Berkeley, CA, USA). The two-tailed Mann-Whitney U-test and the Spearman′s test were used for between-group comparison and correlations, respectively. Data are presented as mean±SEM. A p-value of <0.05 was statistically significant.

Results

Serotonin measurements

In patients and controls, whole-blood serotonin concentrations were within the normal range, as reported previously by the authors′ group (data not shown) [11–13] and others [14]. In contrast, elevated plasma-serotonin concentrations were measured in patients with either severe pulmonary arterial hypertension (38.8±7.3 nmol·L^{-1}) or type-Ia glycogen-storage disease (36.8±11.5 nmol·L^{-1}), as compared to controls (8.8±0.6 nmol·L^{-1}, p<0.001) (fig. 1). Elevated plasma serotonin levels were similar in patients with a past history of fenfluramine intake and patients displaying primary pulmonary hypertension. Similarly, plasma serotonin concentrations were dramatically elevated in the patient with glycogen-storage disease type Ia and severe pulmonary hypertension (113.4 nmol·L^{-1}). Repeated measures in the same individuals did not show significant modifications. In patients and controls, plasma serotonin concentrations were not influenced by age or sex (data not shown), as reported previously [15, 16]. Plasma serotonin did not correlate with any clinical or haemodynamical parameters, such as the 6-min walk test, mean pulmonary artery pressure, the cardiac index, and total pulmonary resistance (data not shown). Platelet serotonin was within the normal range (0.50–5.0 amol·platelet^{-1}) in glycogen-storage disease type Ia (2.41±0.25 amol·platelet^{-1}) and severe pulmonary arterial hypertension (4.36±3.60 amol·platelet^{-1}). There was a trend for lower platelet counts in pulmonary arterial hypertension (140±63×10^9 L^{-1}) as compared to glycogen-storage disease (357±51×109 L^{-1}) or controls (332±51×10^9 L^{-1}).

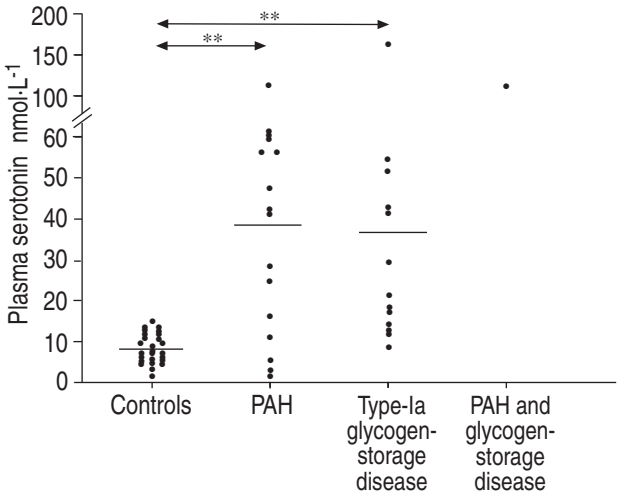

Fig. 1. – Plasma serotonin concentrations in primary pulmonary hypertension, type-I glycogen- storage disease and normal healthy controls. The horizontal bar represents mean values. **: p<0.01.

Discussion

A case of severe pulmonary hypertension in a patient with type-Ia glycogen-storage disease is reported. Interestingly, the patient with severe pulmonary arterial hypertension and type-Ia glycogen-storage disease had elevated plasma serotonin concentrations, suggesting that this mediator may play some role in the occurrence of pulmonary arterial hypertension. In order to evaluate whether abnormal

serotonin-plasma levels are a common feature in type-Ia glycogen-storage disease and without overt pulmonary hypertension, this parameter was prospectively measured in 13 consecutive patients referred to the authors' hospital for this condition. Demonstration of elevated plasma serotonin in these individuals, in the absence of pulmonary arterial hypertension, suggested that elevated plasma serotonin is a characteristic of type-Ia glycogen-storage disease. However, most patients will not develop severe pulmonary vascular disorders, emphasising the argument that additional factors of individual susceptibility may be required to lead to pulmonary vascular involvement. Indeed, it has been previously suggested that individual susceptibility to serotonin explains the occurrence of pulmonary arterial hypertension in a minority of patients exposed to an established risk factor [2]. For instance, only a minority of patients using fenfluramine derivatives, appetite suppressant drugs that impair platelet storage of serotonin and elevate its plasma levels, developed pulmonary arterial hypertension, indicating that drug-induced abnormal serotonin handling was one event in a multistep process leading to severe pulmonary arterial hypertension in susceptible individuals [2, 17]. Recent studies have shown that patients with a predisposition for developing primary pulmonary hypertension may manifest early changes in pulmonary hypertension when studied by stress echocardiography [18]. Further studies are needed to evaluate whether patients with type-I glycogen-storage disease and pulmonary hypertension may have abnormal stress echocardiography.

Current results concerning the possible role of serotonin in the setting of pulmonary hypertension complicating the course of type-I glycogen-storage disease are purely descriptive and direct evidence that serotonin is causally related to pulmonary hypertension cannot be provided. Moreover, the exact mechanisms leading to the increase in plasma serotonin in type-I glycogen-storage disease remain unclear. Blood serotonin is produced by the enterochromoffin cells of the intestine. Virtually all blood serotonin is stored in the platelets and free serotonin is rapidly metabolised by the endothelial monoamine oxidase in the liver and the lung. Increased plasma concentrations of free serotonin in type-Ia glycogen-storage disease might be the result of excessive production by the gut and/or decreased endothelial metabolism and/or impaired ability of the platelets to store large amounts of serotonin [11]. The authors also confirm that severe pulmonary arterial hypertension is associated with elevated serotonin plasma levels. As was shown previously, these raised plasma concentrations were not the mere consequence of pulmonary arterial hypertension, as they remained elevated after successful lung transplantation [11]. To the best of the authors' knowledge, no recurrence of pulmonary arterial hypertension has been described after lung transplantation, suggesting that elevated plasma serotonin is not sufficient to induce a relapse of the disease in the years following surgery [11]. Nevertheless, further long-term studies are needed to firmly establish this point. In addition to chronic serotonin exposure, abnormal native pulmonary endothelial cells (increased endothelin-1, decreased prostaglandin synthase or nitric oxide synthase) or smooth muscle cells (abnormal potassium channels, abnormal serotonin transporter) may be critical in the development of pulmonary arterial hypertension [19].

Table 3 shows that six out of the seven reported cases of severe pulmonary arterial hypertension complicating the course of type-I glycogen-storage disease occurred in the second or third decade of life, suggesting that long-term metabolic abnormalities may be necessary to lead to pulmonary hypertension. The only case occurring before the age of 10 yrs was in a young female with atrial-septal defect of the secundum type and type-Ia glycogen-storage disease [8]. Interestingly, neither atrial-septal defect nor type-Ia glycogen-storage disease usually lead to severe pulmonary hypertension in the first decade of life. Therefore, these conditions could have cumulative negative effects with a faster progression of the pulmonary vascular disease [8, 20].

The patient in the present study had a history of porto-caval shunt without portal hypertension. Indeed, until ~1984, some authors proposed porto-caval shunts in type-I glycogen-storage disease to improve the patient's growth status [21]. Table 3 shows that three out of the seven published cases of type-I glycogen-storage disease with pulmonary arterial hypertension had a history of shunt operation, suggesting that substances of intestinal origin normally metabolised in the liver had significantly influenced the course of the disease [5, 7]. Serotonin, a product of the enterochromoffin cells of the gut, is one of these substances. Under normal conditions, the pulmonary vascular bed is not exposed to excessive serotonin levels, because of its position as a second filter located downstream of the liver, and because of the ability of platelets to store large amounts of serotonin [11]. Since vasoconstrictive pulmonary hypertension is uncommon in patients with portosystemic shunts [22], one or more additional factor(s) of individual susceptiblity is (are) believed to be necessary to develop severe pulmonary hypertension after a shunt operation. In order to evaluate whether a simple porto-caval shunt may induce detectable elevation in circulating serotonin concentrations, whole blood and plasma serotonin concentrations were measured in a group of nine patients with porto-caval shunts. In this population, whole blood and plasma serotonin concentrations were within the normal range (unpublished data). Therefore, elevated plasma serotonin concentrations detected in the patient with a patent porto-caval shunt, described in the present report, could not be explained by the presence of a porto-caval shunt.

The patient presented with multiple adenomas of the liver. With longer survival of patients with type-Ia glycogen-storage disease, cases with hepatic tumours (mostly adenomas) have been increasingly demonstrated [23]. Adenomas are also known to be associated with the use of oral contraceptives, a condition known to increase oestrogen in the systemic circulation [24]. Interestingly, sex is regarded as a definite risk factor of primary pulmonary hypertension with a female:male ratio exceeding 1:1.7 in large cohorts,

Table 3. – Pulmonary hypertension (PH) and type-Ia glycogen-storage disease: review of the litterature

First author (Ref. no.)	Age at diagnosis of PH yrs	Sex	Shunt operation	Intracardiac shunt	Hepatic tumors	SPAP (mPAP) (kPa)	Evolution	Pathology (small pulmonary arteries)
PIZZO [5]	16	F	Yes Porto-caval shunt at 12 yrs	No	Yes Focal nodular hyperplasia	ND	CPA Death	Intimal fibrosis Medial hypertrophy Plexiform lesions
FUROKAWA [6] HAMAOKA [7]	12	F	No	No	No	ND	RHF Death (1 month after PH diagnosis)	Fibrous occlusion Plexiform lesions
HAMAOKA [7]	16	M	Yes Intestinal vein/inferior vena cava at 10 yrs	No	No	120	RHF Death (2 months after PH diagnosis)	ND
BOLZ [8]	4	F	No	Yes Atrial septal defect of the second type	No	72 (6.92)	RHF Death (4 years ofter PH diagnosis)	Intimal fibrosis
OHURA [9]	21	F	No	No	No	90	RHF Death (1 week after PH diagnosis)	ND
KISHNANI [10]	24	F	No	No	No	115	RHF Death (8 months after PH diagnosis)	ND
HUMBERT [2]#	25	M	Yes Porto-caval shunt at 8 yrs	No	Yes Hepatic adenomas	100 (8.65)	Alive NYHA II (4 years after PH diagnosis)	ND

F: female; M: male; CPA: cardio-pulmonary arrest; ND: not determined; NYHA: New York Heart Association; RHF: right heart failure; SPAP: systolic pulmonary artery pressure; mPAP: mean pulmonary artery pressure. #: data from present study.

indicating that hormonal influences play a part in these pulmonary vascular diseases [25]. Similarly five out of the seven cases of severe pulmonary hypertension, complicating the course of type-Ia glycogen-storage disease, occurred in females (table 3).

The occurrence of severe pulmonary arterial hypertension in patients with type-I glycogen-storage disease could be due, at least in part, to endothelial cell dysfunction secondary to the metabolic disturbances characteristic of this condition, such as marked hyperlipidaemia [26]. It must be stressed, however, that hyperlipidaemia is not a recognised cause of pulmonary vascular diseases [2] and that patients with type-I glycogen-storage disease appear to be less susceptible to the damaging effects of hyperlipidaemia than the general population [26]. Thrombosis may also play a role in the development or progression of pulmonary arterial hypertension in patients with type-I glycogen-storage disease, as previously suggested with regard to primary pulmonary hypertension [27]. Indeed, TAKI *et al.* [28] have shown that thrombus formation may be a result of abnormal platelet adhesion to subendothelium in type-I glycogen-storage disease [28]. However, when available, pulmonary histology did not show marked pulmonary thrombosis in patients displaying severe pulmonary arterial hypertension and type-I glycogen-storage disease (table 3). Moreover, there was no evidence of thromboembolic disease in the patient reported in the present study.

Familial and sporadic primary pulmonary hypertension can be caused by mutations in receptor members of the transforming growth factor-β family (BMPR2 and ALK1) suggesting that dysfunctional transforming growth factor-β signalling could lead to abnormal proliferation of pulmonary vascular cells [29, 30]. The patient with type-Ia glycogen-storage disease and pulmonary arterial hypertension was sequenced for all BMPR2 and ALK1 exons. No sequence variants were found. The gene responsible for type-Ia glycogen-storage disease (glucose-6-phosphatase deficiency) has been cloned on the long arm of chromosome 17r (17q21) [31]. Several mutations have been detected in the coding sequence of the gene in type-Ia glycogen-storage disease, thus illustrating the genetic heterogeneity of this condition [31]. Further studies are needed to investigate a possible gene linked to pulmonary hypertension in the same chromosomic region.

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