Physiol. Res. 55 (Suppl. 2): S103-S108, 2006

Individualized Workup - a New Approach to the Biochemical Diagnosis of Acute Attacks of Neuroporphyria

N. SCHOENFELD[1,2], R. MAMET[1]

[1]*Porphyria Reference Laboratory, Rabin Medical Center, Beilinson Hospital, Petah Tikva,*
[2]*Sackler, Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

Received June 14, 2005
Accepted October 24, 2006

Summary

Porphyria experts concur that acute attacks of AIP, VP and HCP, are invariably associated with increases in urinary PBG. Reports differ, however, as to the amount of increase indicative of an acute attack. Some authors consider excretion of at least 25-fold the upper level of normal, as indicative, whereas others regard a 10-fold or even a 2-fold increase, as sufficient indication. An additional diagnostic difficulty arises from the fact that in many individuals known to have inherited one of the acute porphyrias, PBG is persistently raised also during remission. It may be markedly elevated even in asymptomatic carriers. In the absence of a universally accepted standard for interpreting PBG results, attribution of neurovisceral or neuropsychiatric symptoms in porphyrics to an acute attack of porphyria rather than to other causes, depends largely on clinical assessment. The aim of this work was to identify reliable criteria, which will enable establishing or excluding an acute attack, on a biochemical basis. The study summarizes and interprets data obtained during classical neurovisceral acute attacks and latent phases in 20 patients (10 with AIP, 6 with VP, and 4 with HCP). Calculated increases in urinary PBG, with the upper limit of normal excretion, (8.8 μmol/24 h), defined as 100 %, revealed an overlap between values in the acute and latent phases, (1 to 18.5-fold and 2.3 to 51-fold, respectively). This overlap indicates that the workup in each case needs to be individualized. We achieved this goal, by using another method of calculation, in which the PBG value measured during an acute attack in a particular patient was divided by the PBG value measured in that patient's latent phase. Increases of 2.3 to 50.5-fold were obtained, leading to the conclusion that any increase, calculated as above, of 2.3-fold and higher, may be taken as indicative of an acute attack. An additional finding, demonstrated in the study, which might be useful for supporting the diagnosis of an acute attack, is the distinct emission peak observed at 404/621 nm, in the plasma fluorometric scan of AIP and HCP patients, during an acute attack. We conclude that comparison of the urinary PBG level and plasma fluorometric scan in the acute phase to those of the latent phase in the individual patient is the key to correct, accurate and reliable biochemical diagnosis of an acute attack in a patient previously diagnosed as a porphyric. The additional tests required for confirming a patient's first acute attack, having no data to compare with, are discussed.

Key words

Porphobilinogen (PBG) • Porphyria • Acute attack • Biochemical diagnosis

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Introduction

Acute intermittent porphyria (AIP), variegate porphyria (VP), and hereditary coproporphyria (HCP) are all classified as acute porphyrias because they share identical neurovisceral crises. Acute attacks are invariably associated with increased urinary porphobilinogen (PBG). This finding currently serves as the basis for establishment of the diagnosis. Reports differ, however, as to the amount of increase signifying an acute attack. According to the textbooks of Cecil and Harrison (Desnick 2005, Anderson 2004), during an acute attack, PBG excretion is generally in the range of 50–200 mg/day (221-884 μmol/24 h), a value which is 25-100-fold the upper limit of normal. According to the European Porphyria Initiative website (European Porphyria Initiative) "in most patients with an attack of acute porphyria, PBG concentrations are at least 10 times the upper limit of normal". Some authors, however, regard an increase of 5-fold (Mustajoki and Nordmann 1993) or even 2-fold (Tefferi *et al.* 1994) as sufficient indication.

An additional diagnostic difficulty is that in many individuals known to have inherited one of the acute porphyrias, PBG is persistently raised also during the latent phase (Ackner 1961). It may be significantly elevated even in asymptomatic carriers. An increase of 50 fold the upper limit of normal (440 μmol/24 h) was reported in an asymptomatic AIP patient who never had an acute attack (Goldberg 1954).

Thus, in the absence of a universally accepted standard for interpreting PBG results, attribution of neurovisceral or neuropsychiatric symptoms in porphyrics to an acute attack, depends largely on clinical assessment.

This retrospective work was aimed at defining reliable criteria, which will enable establishment or exclusion of an acute attack, on a biochemical basis.

Patients and Methods

Patients

In the following work we summarize and interpret data obtained during acute and latent phases in 20 patients. Patients included in the study were only those who experienced acute attacks that presented with the classical symptoms of abdominal pain with or without peripheral neuropathy or psychiatric symptoms (Table 1), and treated successfully with either glucose, or Normosang or both. All were referred to our laboratory, which serves as a national reference laboratory for the biochemical diagnosis of porphyria, from hospitals and clinics throughout the country. The specimens of urine, feces, and blood reached the lab within 4 hours from everywhere in the country, and were accompanied by information sheets recording the relevant data pertaining to past and present clinical symptoms and drug therapy.

Porphyria diagnosis

AIP was diagnosed in ten of the patients, of whom in nine, decreases of 40–60 % in the activity of porphobilinogen deaminase (PBGD) were observed, and in one (patient no 1), presenting with highly increased urinary PBG and aminolevulinic acid (ALA), normal PBGD activity was demonstrated in both the acute and the latent phases. In six patients, diagnosis of variegate porphyria was established on the basis of increased fecal protoporphyrin and coproporphyrin, reversal of the normal ratio of fecal coproporphyrin III/I, and a distinct peak at 404/628 nm in the fluorometric plasma scan. In four patients, hereditary coproporphyria was identified due to markedly increased fecal coproporphyrin and a reversal of the normal fecal coproporphyrin III/I ratio.

Methods

PBG and ALA in 24-h urinary collections were determined by the method of Buttery and Stuart (1991) and Berko and Durko (1972), respectively. Urinary and fecal porphyrins were measured by HPLC, as previously described (Schoenfeld *et al.* 1995). PBGD activity in erythrocytes was measured according to Magnussen et al (1974). Fluorescence emission spectroscopy of plasma was carried out as described by Long *et al.* (1993), with minor modifications.

Results and Discussion

During acute attacks, increases in PBG, higher than 25-fold the upper limit of normal, were observed in only seven of the 20 patients (Table 1). Defining the cutoff point as a 10-fold increase, could have resulted in a misdiagnosis of an acute attack in seven patients in whom lower elevations were measured during the acute attack, as well as an erroneous diagnosis of an acute attack in four patients in the latent phase exhibiting PBG values increased by more than 10-fold (Table 1). It is therefore not surprising that the increase above the generally accepted upper limit of normal PBG excretion (8.8μmol/24h), showed an overlap between the results

obtained in the latent and in the acute phases, namely 1–18.5 times and 2.3–51 times the upper limit, respectively. This overlap demonstrates the need to relate to the workup in each patient on an individual basis.

To achieve this objective we employed a different method of calculation, in which the PBG defined as 100 % differs from patient to patient and reflects in each case the value measured during that individual's latent phase rather than an arbitrarily chosen universal value of the upper limit of normal. The increase in PBG during an acute attack was then calculated in each of the 20 patients by dividing the PBG value measured during an acute attack by the PBG value measured in that patient's latent phase. Increases of 2.3- to 25-fold were obtained (Table 1) leading us to conclude that an increase of 2.3-fold, calculated as above, is the minimal increase that can be regarded as indicative of an acute attack.

Table 1. Urinary PBG and uroporphyrin during latent and acute phases in patients with acute porphyrias

Patient	Age (yrs)	Symptoms during acute attack	PBG Latent		PBG Acute			URO Latent	URO Acute
			µmol/ 24h	Fold × Normal	µmol/ 24h	Fold × Normal	Fold × Latent	Fold × Normal	Fold × Normal
Acute intermittent porphyria									
1 (f)	21	# ^ *	163.5	18.5	450.8	51	2.76	2.7	104
2 (f)	30	# ^	92.8	10.5	287.3	32.5	3.1	3	178
3 (m)	31	# ^	97.3	11	225.4	25.5	2.3	9.0	131
4 (f)	17	# ^	8.8	1	154.7	17.5	17.6	10.0	88
5 (f)	16	# ^	30.9	3.5	287	32.5	9.3	----	----
6 (f)	48	# ^	12.4	1.4	37.1	4.1	3.0	3.3	15
7 (f)	37	# ^	43.8	4.95	132.6	15	3.0	10.5	16
8 (f)	25	# ^	17.7	2	53.0	6	3.0	----	----
9 (f)	19	# ^	123.8	14	282.9	32	2.3	6.8	600
10 (m)	17	#	35.4	4	150.3	17	4.25	----	----
Variegate porphyria									
11 (f)	53	# ^ *	13.3	1.5	265.2	30	19.9	2.6	136
12 (f)	63	# ^	8.8	1.0	79.6	9	9	2.7	97
13 (f)	50	# *	11.0	1.25	44.2	5	4	----	----
14 (f)	19	#	10.6	1.3	64.5	7.3	6.1	----	----
15 (m)	13	# *	8.8	1.0	221.0	25.0	25.0	8.0	600
16 (f)	43	#	8.8	1.0	27.4	3.1	3.1	----	----
Hereditary coproporphyria									
17 (m)	30	#	9.3	1.0	20.3	2.3	2.2	----	----
18 (m)	28	# ^ *	17.7	2.0	154.7	17.5	8.7	4.0	70
19 (m)	19	# ^	15.5	1.75	95.0	10.7	6.1	----	-----
20 (m)	31	# ^	19.9	2.25	110.5	12.5	5.6	5	200

PBG: porphobilinogen; normal value: <8.8 µmol/24h; URO: uroporphyrin; normal value: <36 nmol/24 h
#, abdominal pain: ^, peripheral neuropathy; *, psychiatric symptoms

Table 2. PBG Urinary Level: Calculation per 24h versus calculation per creatinine

Patient	Phase	Creatinine* mmol /24 h	PBG μmol/24h	PBG μmol/10mmol creatinine
1	Latent	2.65	75	283
2	Latent	5.3	198.4	374
3	Latent	21.2	35.3	17
4	Acute	21.2	264.6	125

*Normal value: 5.3-15.9 mmol/24 h

We find it noteworthy, that it's crucial to have data concerning the creatinine excretion of the patients. In case of emergency, PBG should be determined in a urine sample rather than in a 24 h collection, and calculated per creatinine. A highly increased or decreased creatinine excretion may affect the results, and as a consequence, the interpretation. Table 2 describes data of four of the patients with abnormal creatinine excretion. As shown in the Table, in the first two patients creatinine excretion was low. Consequently, the values of PBG calculated per creatinine were 1.8 and 3.8 fold the true PBG values, measured in 24 h urinary collections. In the other two patients, whose creatinine excretion was highly increased, the calculated level of PBG per creatinine was only half the true level measured per 24 h. It is therefore concluded that PBG results when calculated per creatinine should be carefully interpreted taking into account the ratio between results per 24 h urinary collection and per creatinine excretions, in the individual patient.

Another test, which was found to be useful in supporting a diagnosis of an acute attack, is the plasma fluorometric emission scan. It was previously reported that in patients with lead poisoning (acquired porphyria) during the acute phase a distinct peak in the emission spectra, at 404/635 nm was observed (Mamet *et al.* 2001). The peak was much less pronounced in the non-acute phase. A similar phenomenon was observed in the AIP and HCP patients studied. Representative spectra revealing significantly higher peaks at 404/621 nm during acute attacks, are shown in Fig 1.

On the basis of the above findings, we conclude that comparison of the urinary PBG level and plasma fluorometric scan in a symptomatic phase to those in the latent phase in the individual patient is the key to correct, accurate, and reliable biochemical diagnosis or exclusion of an acute attack in a patient previously diagnosed with porphyria.

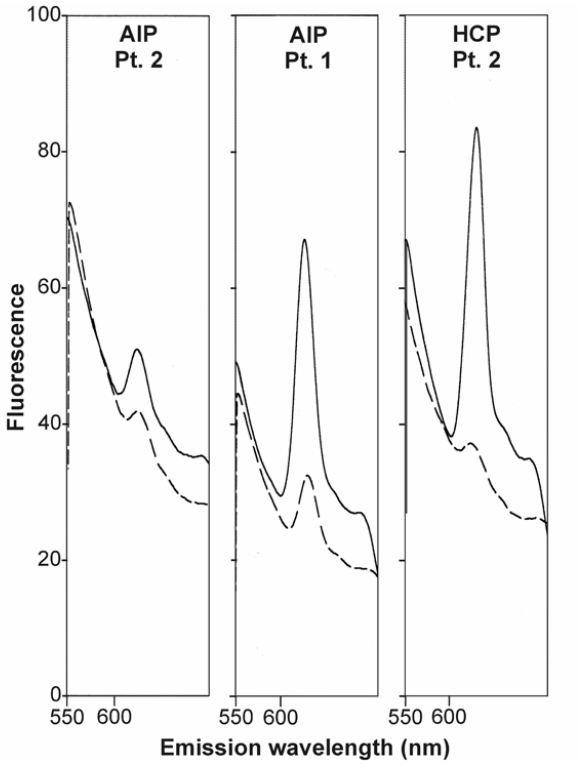

Fig. 1. Plasma fluorescence emission spectra of porphyric patients in the latent and acute phases.
Plasma from three patients during latent (_ _ _) and acute (___) phases, was diluted in saline (1:10). Each sample was subjected to fluorescence emission spectroscopy with the fixed excitation wavelength at 404 nm.

In a new patient with suspected attack of acute porphyria, obviously there are no previous data for comparison. Establishing the diagnosis of an acute attack is therefore much more complicated and requires additional tests, especially if the elevations in urinary PBG are moderate, with values lower than 88.4μmol/24h (10-fold increase). An increase of this order could be primary in a porphyria patient, but could also be secondary to other clinical or therapeutic conditions unrelated to porphyrias, such as liver diseases (MedlinePlus) or treatment with imipenem (Verstraeten

et al. 1992) as well as with other drugs (MedlinePlus). It is therefore crucial to determine first whether the patient indeed has porphyria and that by defining the type of porphyria. Only once the diagnosis of porphyria is established is it pertinent to consider attributing the symptoms to an acute attack.

In order to define the type of porphyria it is necessary to perform all of the following inseparable tests: 24h urinary ALA, PBG, uroporphyrin and coproporphyrin, fecal protoporphyrin, total coproporphyrin, and coproporphyrin III/I ratio, plasma fluorometric scan and erythrocytes PBGD activity.

The criteria used for defining VP and HCP during acute attacks are basically similar to those acceptable during the latent phase. However, diagnosing AIP during acute attacks, very often poses a real problem since PBGD during acute attacks is induced and its measured activity is therefore normal or even increased (Kostrzewska and Gregor 1986).

While looking for a factor which might help us identify AIP during acute attacks we found out that urinary uroporphyrin rather than coproporphyrin may serve as an indicator of an acute attack. Uroporphyrin was found to be elevated by up to 9 fold the upper limit of normal in the latent phase of AIP patients (not shown) while increases of 15-600 fold (Table 1) were demonstrated in the acute phase. It is interesting to note that the marked increase in the urinary uroporphyrin during acute attacks is a phenomenon shared also by HCP and VP, and is not specific to AIP only (Table 1). In view of the finding that the increase in urinary coproporphyrin was less pronounced than that of uroporphyrin (not shown) and the well known fact that coproporphyrinuria might be non-specific, secondary to many clinical conditions unrelated to porphyria (Lamon 1977), we recommend adding uroporphyrin rather than coproporphyrin to the diagnostic markers of acute attacks. Therefore, once either AIP, HCP or VP is defined in a new patient, confirmation of an acute attack will depend on his UPS: Uroporphyrin – which should be increased by at least 15 fold the upper limit of normal, Porphobilinogen – which should exceed at least 20.3 μmol/24h, and an abnormal fluorometric plasma Scan.

However, the interpretation will always be more reliable in a patient previously diagnosed with porphyria, with a well documented data on values of URO, PBG and Scan as a start point for evaluation of future suspected attacks.

It is our hope that the approach of the individualized workup, presented in this work will contribute to avoidance of both negative and positive false diagnoses.

Acknowledgement

The excellent technical assistance of R Mevasser is gratefully acknowledged.

References

ACKNER B, COOPER JW, GRAY CH, KELLY M, NICHOLSON DC: Excretion of porphobilinogen and 5-aminolaevulinic acid in acute porphyria. *Lancet* **1**: 1256-1260, 1961.

ANDERSON KE. The porphyrias. In: *Cecil's Textbook of medicine.* L GOLDMAN, D AUSIELLO (eds): 22 ed Philadelphia, PA: WB Saunders Co., 2004, pp: 1292-1300.

BERKO G, DURKO I: A new possibility for the demonstration of amino laevulinic acid in urine on the basis of Mauzerall Granick method. *Clin Chim Acta* **37**: 443-447, 1972.

BUTTERY JE, STUART S: Measurement of porphobilinogen in urine by a simple resin method with use of a surrogate standard. *Clin Chem* **37**: 2133-2136, 1991.

DESNICK RJ: The Porphyrias In: *Harrison's principles of internal medicine*. DL KASPER, AS FAUCI, E BROUNWALD, SL HAUSER, JL JAMSON, TR HARRISON, (eds. 16 ed New York: McGraw Hill 2005, chapter 337.

EUROPEAN PORPHYRIA INITIATIVE (EPI). http://www.porphyria-europe.com, 2005.

GOLDBERG A: Renal clearance of endogenous porphobilinogen in man. *Lancet* **267**: 1095-1097, 1954.

KOSTRZEWSKA E, GREGOR A: Increased activity of porphobilinogen deaminase in erythrocytes during attacks of acute intermittent porphyria. *Ann Clin Res* **18**: 195-198, 1986.

LAMON JM: Clinical Aspects of porphyrin measurement other than lead poisoning. *Clin Chem* **23**: 260-263, 1977.

LONG C, SMYTH SJ, WOOLF J, MURPHY GM, FINLAY AY, NEWCOMBE RG, ELDER GH: Detection of latent variegate porphyria by fluorescence emission spectroscopy of plasma. *Br J Dermatol* **129**: 9-13, 1993.

MAGNUSSEN CR, LEVINE JB, DOHERTY JM, CHEESMAN MO, TSCHUDY DP. A red cell enzyme method for diagnosis of acute intermittent porphyria. *Blood* **44**: 857-868, 1974.

MAMET R, SZTERN M, RACHMEL A, STAHL B, FLUSSER D, SCHOENFELD N: Lead poisoning: A new biochemical perspective on the differentiation between acquired and hereditary neuroporphyria (Technical Brief) *Clin Chem* **47**: 1710-1713, 2001.

MEDLINEPLUS, GRECO FA: www.nlm.nih.gov/medlineplus/ency/article/003596.htm, 2004.

MUSTAJOKI P, NORDMANN Y: Early administration of heme arginate for acute porphyria attacks. *Arch Intern Med* **153**: 2004-2008, 1993.

SCHOENFELD N, MAMET R, DOTAN I, SZTERN M, LEVO Y, ADERKA D: Relation between uroporphyrin excretion, acute attack of hereditary coproporphyria and successful treatment with haem arginate. *Clin Sci*; **88**: 365-369, 1995.

TEFFERI A, COGAN JP, SOLBERG LA Jr: Acute porphyrias: diagnosis and management. *Mayo Clin Proc* **69**: 991-995, 1994.

VERSTRAETEN L, LEDOUX MC, MOOS B, CALLEBOUT B, CORNU G, HASSOUN A: Interference of Tienam in colorimetric determination of 5-aminolevulinic acid and porphobilinogen in serum and urine. *Clin Chem* **38**: 2557-2558, 1992.

Reprint requests

Nili Schoenfeld, Porphyria Reference Laboratory, Rabin Medical Center, Beilinson Hospital, 49100 Petah-Tikva, Israel. Fax: +972-3-9377768. E-mail: nschoenfeld@clalit.org.il