



## “PORPHYRIAS AND ASSOCIATED PATHOLOGIES. BIOCHEMISTRY AND MOLECULAR BIOLOGY”

To start with, we wish to thank Professor Dr. Raymond Wegmann “The Founding President” and Professor Dr. Jean-Michel Maixent, “The President”, Editors-in-Chief of *Cellular and Molecular Biology*™ for their generous invitation to become Guest- Editor and Co-Guest Editor of this special theme issue on “*Porphyrias and associated pathologies. Biochemistry and Molecular Biology*”, which will be published in two Volumes, Part I, appearing now, February 16 th, 2009 and the next, Part II, scheduled for June 2009.

Although *Cellular and Molecular Biology*™ has already published three others special issues on this subject, one in 1997, and two in January and December 2002, ... “to have another issue on “*Porphyrias and Porphyrias*” is not too much, since it belongs to one of the modern problems for which orphan drugs are requested”... (quoted from Professor Dr. Raymond Wegmann, *Cellular and Molecular Biology*™, 2002, **48**, 823).

We also wish to thank Mr Mourad Fares, Executive Editor of CMB, for his support, composition and editing of the papers.

The articles put together in Part I of this theme issue, come from the highest experts in the world in this field, biologists, physicians, chemists, physics, all of them leading porphyrinologists, which will assure, will make Part I as well as the coming Part II, a highly important reference issue for the future. Therefore we are also deeply thankful to all the authors for their contributions and most important their collaboration in making possible the production of this theme CMB issue.

“Porphyrias and Porphyrias”, since the middle of the 50’s, porphyrinologists from all over the world have been meeting at least once or later more than once, mostly in Europe and USA, to discuss about the latest innovative results on the biochemistry, molecular biology, regulation and functional mechanisms of the enzymes involved at each step of the porphyrins biosynthesis, which have been isolated, cloned and even chrystalized, and its related diseases: the Porphyrias.

In 1997, Professor Robert Aquaron, attempted to list a number of the International Porphyrins and Porphyrias Meeting, mostly hold in Europe, since 1955, until then. However the Gordon Research Conferences, started in 1968, occurring in USA, every two years ( about 15) were just mentioned as well as the Tetrapyrrole Discussion Group Meetings, gathering somewhere in Europe, more often in UK, every nine months since 1967 (by then they were about 20 meetings).

Porphyrins and Porphyrias, certainly, one of the most important and endless theme for discussion. Some of us have had the honour and pleasure of having been associated, known and being personally involved in Porphyrins and Porphyrias research for nearly 50 years by now, also having had the pionners in this field as our mentors, something to celebrate, so we will, after reading this special issue of CMB.

Porphyrins synthesis is one of the most fundamental attributes of all living cells.

Central to the fundamental processes of photosynthesis and respiration, are chlorophylls and haem, respectively, which are porphyrins, that is the reason why, Lemberg and Ledge in 1949, coined the expression “*porphyrins are the pigments of life*”, could it be anything more important than that?.

We would wish to partially quote one of us (Batlle, CMB™, 2002, **48**, 823), when saying that porphyrins are unique and intriguing molecules, historically having a geologic and a biologic medical chapter. The former going back many millions of years, when the formation of porphyrin-like compounds and porphyrins was contemporary with the development of life on earth. The latter started at the beginning of nineteen century when iron-free hematin was obtained by Scherer in 1941, after treating dried blood with concentrated sulphuric acid. This pigment was later purified by Thudichum in 1867, who named it “*cruentine*” and described for the first time its “*splendid blood-red*” fluorescence. The term “*porphyrin*” was soon after coined by Hoppe Seyler in 1871.

As already stated, porphyrin biosynthesis is one of the most fundamental attributes of all living cells. Classical isotope tracer studies from the laboratories of Shemin, Rimington, Granick and Neuberger, identified the precursors and intermediates in the haem biosynthetic pathway marking the beginning of a whole new field of research in the biogenesis of these pigments. Then, most of the eight enzymatic steps involved were identified. Today all the enzymes have been cloned and sequenced and most of them have also been crystallized.

The porphyrin pathway is very finely controlled. In most tissues and species, Aminolevulinic Acid Synthetase (ALA-S) is the regulatory enzyme. Regulation occurs by feedback inhibition of ALA-S, so haem deficiency, owing to blocking the pathway at some step, as it happens in the Porphyrrias, releases this inhibition. The term "*porphyria*", gradually emerged after Stokvis(1889) reporting the death of an elderly woman, excreting dark red urine after having received sulfonal.

Human porphyrias are specific inherited or acquired defects, each representing a partial failure of one of the seven enzymes beyond ALA-S and they are characterised by a typical excretion pattern of porphyrin intermediates.

We would like to recall that porphyrins are the only photosensitizers synthesized in the cells and the best examples of these endogenous sensitizers are the porphyrin intermediates formed and accumulated in the cutaneous porphyrias, producing the characteristic skin photosensitization.

Photodynamic Therapy (PDT) is a promising new modality of cancer treatment, which involves the combination of, a photosensitizing agent, which is taken up selectively and retained by tumoural cells, and light of an appropriate wavelength. Separately, each of these factors is harmless by itself, though, when combined, in the presence of oxygen, cytotoxic reactive oxygen species are produced, leading to irreversible cellular damage, causing cell death and tumour destruction.

After either exogeneous administration or endogenous synthesis, porphyrins finally accumulate in higher proliferative cells. Light energy absorbed by the photosensitizer (PS) can produce fluorescence. The tumour localizing properties of the PS have been extensively employed for the Photodetection (PD) and diagnosis, as well as for the PDT of tumours.

In humans, mutations in the Protoporphyrinogen oxidase (PPO) gene, the enzyme catalyzing the penultimate reaction in heme synthesis, resulted in decreased PPO activity leading to Variegate Porphyria (VP), a dominantly inherited disorder characterized by photosensitive skin lesions and a propensity to develop neurovisceral crisis. In the paper "*Peroxidase activity of cytochrome c facilitates the Protoporphyrinogen oxidase reaction*", Mark Shepherd and Harry Dailey, have very elegantly demonstrated that given the cellular location of PPO and the abundance of Cyt.c in the intermembrane space of mitochondria, generation of free radicals, through the peroxidase activity of Cyt c, might potentially impact on heme synthesis *in vivo*, particularly in conditions of low oxygen or hypoxia.

In Hereditary Coproporphyria (HCP), another mixed Porphyria, mutations in the gene of Coproporphyrinogen Oxidase (CPO), lead to reduced CPG activity. Clinical symptoms are rare before puberty and are mostly neurological and less commonly cutaneous. Caterina Aurizi and coworkers, in their paper "*Four Novel Mutations of the Coproporphyrinogen III Oxidase Gene*", have characterized 4 novel mutations and one already described in 5 Italian unrelated families.

The clinical manifestation of porphyrias are often associated with exposure to precipitating agents, including polyhalogenated aromatic hydrocarbons, alcohol abuse, stress, estrogens ingestion, iron overload and infection with Hepatitis C virus (HCV), less frequently, Hepatitis B virus (HBV) infection with the Human Immunodeficiency virus (HIV).

It is also known that in some porphyrias, mild to moderate hepatic iron overload plays a key role in its pathogenesis. Hemochromatosis is the commonest cause of primary iron overload and some mutations in the hemochromatosis gene (HFE), associated with hereditary Hemochromatosis, have been found to be more frequent in PCT.

Association of porphyrias with other pathologies, such as diabetes, lupus, leukaemia, Hansen's disease and cancer has been reported. There is also association of porphyrias with the treatments used for other pathologies, such as estrogen-therapy in prostate cancer and hemodialysis in patients with renal failure.

Porphyrias are often multifactorial, therefore, knowledge of all risk or etiological factors in each patient is most important for the management of the disease.

In the paper “*Awareness is the name of the game: Clinical and Biochemical Evaluation of a Case of a Girl Diagnosed with Acute Intermittent Porphyria Associated with Autism*”, A.S. Luder, R. Mamet, I. Farbstein and N. Shoenfeld, reported for the first time, the association of an AIP patient diagnosed at the age of 15 years, with late autism, first presented at the age of 4 years. Most unusual was that her urinary ALA and PBG were normal, even during the crisis, not compatible with symptomatic AIP, which was only established on the basis of a 64% reduction of RBC PBG Deaminase, later confirmed with the finding of a known AIP mutation. It is strongly recommended that porphyria should be considered in children with late autism, mainly in relation to atypical or unexpected reactions to medication.

The association in three patients with AIP and end-stage renal disease (ESRD) has been reported in the paper “*Porphyrin precursors and porphyrins in three patients with Acute Intermittent Porphyrias and ends-stage renal disease under different therapy regimens*” by E. Sardh, D.H.E. Andersson, A. Henrichson and P. Harper. The authors have followed the course of three patients with recurrent attacks of AIP and end-stage renal disease (ESRD). Plasma PBG and porphyrins were considerably increased in the three patients. In a previous study, the mean urinary and plasma PBG/ALA ratio in biochemically active AIP patients with conserved renal function was 2.0 (normal 0.3) and plasma porphyrin levels were normal (< 10 nmol/L). In this study they show that the progression to ESRD was paralleled by an increase in urinary and plasma PBG/ALA ratio to reach levels above 6 and higher. Plasma porphyrin increased to levels above 1000 nmol/L leading to cutaneous lesions resembling PCT

The development of kidney failure was a devastating complication in these AIP patients with chronic active disease, leading to unavoidable deterioration of peripheral veins, progression of peripheral neuropathy, dialysis treatment and secondary cutaneous lesions. The clinical course could not be reversed by medical treatment in any of the cases. The combined liver and kidney transplantation is now being considered as a final therapeutic option.

Porphyrias are pharmaco and toxicogenetic diseases and the N-methyl-diethyl-aspartate (NMDA) receptor has been reported to play a key role in several acute and chronic neuropathologic syndromes. ALA accumulates in acute porphyrias due to a deficiency in the heme biosynthetic pathway. Considering that glutamate uptake

inhibition caused by ALA could be by one of the reasons conducing to porphyric neuropathy, it was of interest to evaluate the effect of porphyrinogenic agents on NMDA glutamatergic system. In the paper “*Glutamatergic system: another target for the action of porphyrinogenic agents*”, by J. Lavandera, M. Fossatiu, J. Azcurra, A. Batlle and A.M. Buzaleh, the authors have shown that the glutamatergic system appears to be involved in the action of some of the porphyrinogenic drugs in cerebellum.

There is a number of good animal models for erythropoietic protoporphyria (EPP), in particular the clinically relevant  $\text{Fech}^{\text{mlpas}}/\text{Fech}^{\text{mlpas}}$  genetic model, where the hepatobiliary disease represents a constant feature. Similarly, chronic administration of griseofulvin to mice induces pathological changes analogous to those found in patients with EPP-associated liver injury. On the other hand, activation of the epidermal growth factor receptor (EGFR) plays an important role in liver regeneration and resistance to acute injury. However its chronic activation participates in the progression of liver disease, including fibrogenesis and malignant transformation. In the paper “*Epidermal Growth Factor Receptor ligands in murine models for Erythropoietic Protoporphyria: Potential novel players in the progression of liver injury*”, C. Berasain and coworkers, have studied the hepatic expression of the EGFR and its seven most relevant ligands in  $\text{Fech}^{\text{mlpas}}/\text{Fech}^{\text{mlpas}}$  mice bred in three different backgrounds, and in griseofulvin-induced protoporphyria. The authors have observed that the expression of amphiregulin, betacellulin and epiregulin was significantly increased in young EPP mice when compared to aged-matched controls in all genetic backgrounds.

The expression of these ligands was also tested in older BALB/cJ EPP mice, and it was found to remain induced, while that of the EGFR was downregulated. Griseofulvin feeding also increased the expression of the ligands. It was of interest that protoporphyrin accumulation in cultured hepatic AML-12 cells readily elicited the expression of these three EGFR ligands. The authors suggest that protoporphyrin could directly induce the hepatic expression of EGFR ligands, and that their chronic upregulation might participate in the pathogenesis of EPP-associated liver disease.

It is well known that several anaesthetics are unsafe drugs for the Acute Porphyrias, Ana María Buzaleh, M.J. Morán Jiménez, A. Batlle, R. E. de Salamanca and A. Fontanelas, in their paper

*“Induced of Hepatic Aminolevulinic Acid Synthetase activity by Isoflurane in a genetic model for Erythropoietic Protoporphyrria”* have assayed the porphyrinogenic properties of Isoflurane in a mouse genetic model for EPP, to evaluate whether Isoflurane induces alterations in the heme pathway. They have given Isoflurane to wild-type (+/+), heterozygous (+/Fech<sup>m1Pas</sup>) and homozygous (Fech<sup>m1Pas</sup>/Fech<sup>m1Pas</sup>) mice, the activity of ALA-S and PBG-D was measured in different tissues, as well as Heme oxygenase (HO), cytochrome P-450, CYP2E1 and glutathione levels in liver. Porphyrin precursors were measured in 24h-urine samples.

Fech<sup>m1Pas</sup>/Fech<sup>m1Pas</sup> mice receiving anaesthesia show enhanced ALA-S and CYP2E1 activities in the liver and increased urinary excretion of porphyrin precursors. No alterations were found in either PBG-D or HO activities. Diminished glutathione levels suggest that anaesthesia may produce oxidative stress in these animals. They concluded that, Isoflurane induces ALA-S activity and increased excretion of porphyrin precursors in EPP mice. These findings appear to confirm their previous hypothesis indicating that Isoflurane may be an unsafe anaesthetic not only for patients with acute porphyrias but also for individuals with non acute porphyrias.

In another paper on EPP, *“ Excessive erythrocyte PPIX influences the hematological status and iron metabolism in patients with dominant Erythropoietic Protoporphyrria”*, by C. Delaby and coworkers, the authors have written an excellent documented contribution to our knowledge to iron biology in EPP, studying a relative high number of patients, considering the low frequency of this disease. It should also be emphasized that their study has immediate therapeutic consequences in the management of EPP patients, from the point of view of reducing the main symptom, which is its photosensitivity, responsible often for a severe impairment in the quality of life of these patients.

In the Review *“ Congenital Erythropoietic Porphyria: Mutation update and correlations between genotype and Phenotype”*, by Cécile Ged and coworkers, they have challenged the difficult issue to perform a high quality genotype/phenotype analysis in a rare genetic disease such as congenital erythropoietic porphyria (CEP) or Günther’s disease, whereby the heme biosynthesis defect is due to uroporphyrinogen III synthase deficiency. They are providing a very good historical background and the main phenotypic features of the disease, together with an

update of published mutants and genotype/phenotype correlations. General rules concerning the prediction of disease severity are drawn as a guide for patient management as well as therapeutic choices. The phenotypic heterogeneity of the disease is shown in relation with a likely influence of modifying factors, either genetic or acquired.

Hepatoerythropoietic Porphyria (HEP) is the rarest of all Porphyrias, only around 30 cases are described in the world literature so far. Xoana Granata and coworkers, in their paper *“The very first description of a patient with Hepatoerythropoietic Porphyria In Argentina. Biochemical and Molecular Studies”*, report the only Argentinean HEP patient. The case of a 14 years old child with skin manifestations similar to those observed in PCT and CEP, however, biochemical assays, ruled out both CEP and PCT. Although his symptoms were not severe enough to be HEP, the enzymatic activity of URO-D was dramatically reduced to a 5% of normal values and the molecular analysis revealed the presence of two already known different mutations on the patient’s URO-D gene, c.703 C>T and IVS9-1. Each parent carries one of the mutations, but they were absent in the brother. This is the first Argentinean HEP case ever described which appeared in a compound heterozygous form and less residual URO-D activity but associated to a mild phenotype.

In the excellent Review on *“Neurological Manifestations of Acute Intermittent Porphyria”*, E. Pischick and R. Kauppinen, describe very clear the clinical manifestations associated with occasional neurovisceral crises due to overproduction of porphyrin precursors such as ALA which is released from the liver to the circulation. The majority of the acute attacks manifest as a combination of abdominal pain, mild mental symptoms and autonomic dysfunction mainly due to vagal insufficiency. However, both acute peripheral neuropathy and encephalopathy may develop if an acute attack proceeds especially due to administration of porphyrinogenic drugs. Acute porphyric neuropathy is predominantly motor and associates with a history of abdominal pain and dysautonomia, CNS involvement and mild hepatopathy. The pathogenesis of porphyric neuropathy is complex but overproduction of ALA via direct neurotoxicity, oxidative damage, and modification of glutamatergic release may initiate the neuronal damage. Acute encephalopathy manifests as a combination of mental symptoms, seizures, SIADH. Posterior reversible encephalopathy syndrome (PRES), which has been

found in patients' MRI during an acute attack with severe encephalopathy, could explain the pathogenesis of encephalopathy and seizures in AIP. Neurological manifestations are unspecific and careful interpretation of abnormal excretion of porphyrin precursors should be done before the symptoms can be related to inherited acute porphyrias and not to secondary porphyrinuria.

E. Minder, X. Schneider-Yin, J. Sterurer and L. Bachmann in their Review: "A systematic review of treatment options for dermal photosensitivity in erythropoietic protoporphyria", have performed a systematic database search on studies related to treatment of EPP. 25 total relevant studies were retrieved, 16 of them dealing with the application of beta-carotene. Two studies were found on each of the three substances, n-acetyl-cysteine (NAC), cysteine, and dihydroxyacetone/Lawson (henna). In addition, single studies on vitamin C, canthaxanthin and UVB treatment respectively, were located. The total number of patients in the 25 studies was 454, including 337 patients in the various beta-carotene trials. Most studies were published in the 1970's. Only 5 studies were randomized and controlled trials; the rest were either open-label, uncontrolled studies or retrospective case reports. Four of the five well-designed studies suggested lack of efficacy of beta-carotene, NAC and vitamin C. The results of the beta-carotene studies were strongly contradictory and efficacy was inversely correlated with study quality. The authors conclude that the available data are insufficient to prove efficacy of any treatment carried out so far in EPP.

The group of Prague, in the paper "Does Bilirubin level correspond to interaction of c.-3279T>G and A(TA)7TAA Variants in UGT1A1 Gene?" by L.Slachtova and coworkers, attempted to establish the role of c.-3279T>G on hyperbilirubinemia in humans. They have demonstrated, that this variant is strongly associated with affection by the Gilberts syndrome, with OR 34.42. This association signal is probably not only due to strong linkage disequilibrium with the other variant (TA)7,. They correlated bilirubin levels with coincidental occurrence of both variants. Since the occurrence of (TA)7 allele in the Caucasian population is 30-40%, controls with (TA)7/(TA)7 with no signs of hyperbilirubinemia, can not be rejected. Also the variability of bilirubin in an individual can influence the results, which is why they calculated with average bilirubin level obtained from at least three measurements.

The paper "5-Aminolevulinatase: Catalysis of the first step of Heme Biosynthesis"

By Gregory Hunter and Gloria Ferreira, contains an excellent mechanistic review of the catalysis of the first step in heme biosynthesis by ALA-S. They discuss the catalytic reaction within the context of both the recently solved crystal structure of *R. capsulatus*, which is 49% identical to human ALA-S 2, and the intrinsic chemical/enzymological kinetics of the reaction. The paper is very clearly and succinctly written and, indeed, it would add immensely to our knowledge and understanding to the ALA-S catalytic mechanism.

Finally, we expect the papers here presented proved not only information, but will also give the sense of their outstanding contribution to our knowledge in this field, and, again, we wish to emphasize that they will constitute a very important and unique reference issue for the future in this theme.

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