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EDITORIAL

# Administration of a Small, Interfering RNA Molecule: A New Effective Method for Preventing Attacks of Acute Porphyria

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The porphyrias are disorders of the heme biosynthetic pathway. Heme is a very important prosthetic group of several human and mammalian proteins, including hemoglobin, myoglobin, cytochromes, catalases and peroxidases. That's why it is synthesized not only in the bone marrow (75-80 % of the whole) but also in the liver (15-20%), muscles and other organs.[1]

Heme is a tetrapyrrole, e.g. a molecule consisting of four pyrrole rings bound via methine bridges and linked in cyclic fashion. The cyclic tetrapyrroles can chelate several metal ions forming biological pigments of major importance –such as heme (with iron), chlorophyll (with magnesium) or cobalamin (with cobalt). In humans, heme is synthesized through a complex biosynthetic procedure involving eight enzymes. The initial step takes place in the mitochondria, where the amino acid glycine reacts with succinic acid to form δ-aminolevulinic acid (ALA), the first intermediate product of this metabolic pathway. This reaction is catalyzed by the enzyme ALA-synthase (ALA-S). The biosynthetic procedure is then transformed to the cytoplasm, where two molecules of ALA are condensed to form a pyrrole, porphobilinogen (PBG). Through the concurrent action of two different enzymes, four molecules of PBG form the first tetrapyrrole molecule, uroporphyrinogen. In the following steps, the side chains of the tetrapyrrole undergo several changes passing through coproporphyrinogen and protoporphyrinogen to finally form protoporphyrin. An atom of iron is incorporated to the protoporphyrin and so heme is produced. All these steps are catalyzed by four additional enzymes, the last three of them being present in mitochondria. So, the biosynthetic procedure begins and ends in mitochondria, with an interlude occurring in the cytoplasm.[2]

It must be here emphasized that heme biosynthesis in the liver -but not in the bone marrow- is regulated through a negative feed-back mechanism involving the final product, heme. Heme inhibits the synthesis of ALA-S, which is the first and rate-limiting enzyme of the metabolic pathway. When heme is in abundance in the hepatic cell, ALA-S synthesis is decreased and the whole procedure runs slower. When liver heme is depleted, we have the opposite result. Hepatic ALA-S synthesis is also downregulated by excess of glucose (glucose effect). These mechanisms are of major importance in the pathogenesis of the attacks of acute porphyria.

The first medical description of a case of porphyria was made in 1889 by Barent Stokvis, a Dutch professor of Medicine. King George III of England[3], the poet Heinrich Heine and the painter Vincent van Gogh were possibly suffering of some type of porphyria. All porphyrias are inherited disorders due to gene mutations, with the

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exception of porphyria cutanea tarda –the most common type of cutaneous porphyrias– which is an acquired disease. The underlying biochemical defectisdecreased activity of one the last seven enzymes (from the 2nd to the 8th) involved in heme biosynthesis. According to whether the disorder mainly affects heme synthesis in the bone marrow or the liver, the disease is classified as erythropoietic or hepatic porphyria. There are several types of hepatic porphyria –according to the affected enzyme– which differ in their clinical manifestations. Some of them present with acute attacks of several neurologic symptoms while in the intermediate periods –more or less prolonged– the patient is asymptomatic. These are called acute porphyrias. In the other types –as well as in all the erythropoietic porphyrias– the patient has only cutaneous manifestations with periodic exacerbations. These are called non-acute or cutaneous porphyrias.

The gene mutation is a necessary prerequisite, but not per se sufficient for the clinical manifestation of acute porphyria. Among the mutation carriers, 1 in 3 has abnormal laboratory findings and only 1 in 5-10 is symptomatic. The underlying decreased activity of one enzyme results in decreased production of hepatic heme. So, ALA-S synthesis is induced through the negative feed-back mechanism. Increased ALA-S activity results in overproduction of intermediate products prior to the defective enzyme. The heme precursors are useless but harmless in normal concentrations and they are excreted, according to their water solubility, by the urine or feces. However, in increased concentrations they become toxic either for the nervous system (mainly ALA and possibly PBG) or the skin (uro-, copro, and proto-porphyrin). This explains why the clinical manifestations of porphyrias are neurologic and/or cutaneous. For example, if the defective enzyme is the 3rd one –as happens in acute intermittent porphyria, the most common type of acute porphyria– ALA and PBG are overproduced and accumulated and the patient has only neurologic symptoms. Defects of some of the subsequent enzymes may cause overproduction of neurotoxic intermediates and porphyrins and so the patient may have both neurologic and cutaneous manifestations of porphyria.

Attacks of acute porphyria usually occur between the 2nd and 4th decades of life, tending to become rarer or disappear with increasing age. Menopause or development of diabetes mellitus[4] are two possible explanations for this phenomenon. Abdominal pain is the most usual clinical manifestation. It is constant, severe, imitating acute abdomen, although not actually accompanied by peritonism. Vomiting, tachycardia and hypertension usually coexist, while constipation is often persisting during the whole period of the attack. All these manifestations are not related to any visceral pathology but only to autonomic neuropathy. Hyponatremia due to inadequate antidiuretic hormone secretion is a common finding and may be severe. In a considerable proportion of patients, acute visceral symptoms may progress to motor neuropathy presenting mainly as weakness of the proximal limb muscles. In a few cases, neuropathy also involves the respiratory muscles, becoming life-threatening. Sensory changes and seizures may be additional features of the porphyric attack. In two less common types of acute porphyria (variegate porphyria, hereditary coproporphyria) skin lesions with marked photosensitivity may also coexist.

There are several precipitating factors of acute attacks. The most common are prolonged starvation or marked reduction of carbohydrate intake, administration of drugs that either directly induce ALA-S or decrease hepatic heme by increasing liver cytochromes synthesis, over secretion of female hormones –especially progesterone, as in the luteal phase of the menstrual cycle or in pregnancy–, cigarette smoking and stress induced by infection, surgery or psychological factors. However, sometimes attacks occur without a recognizable triggering factor.[5]

Acute porphyria is generally considered as a succession of crises with complete resolution of symptoms among them. However, during the last decades it is recognized that acute porphyria may also have chronic complications. Persisting chronic pain due to an axonal motor polyneuropathy may appear, especially in patients with multiple recurrent attacks. In some cases, it is so severe that leads to depression and anxiety or even suicide attempts. Chronic renal disease of the type of chronic tubulo-interstitial nephropathy or focal cortical atrophy (possibly attributed to ALA nephrotoxicity), mental disorders (hysteria or neurosis) and chronic hypertension are other known chronic complications. Furthermore, the risk of hepatocellular cancer is considerably higher than in the general population.[1]

Management of acute attacks is both symptomatic and etiologic. Relief of pain and the other symptoms is limited by the fact that several common drugs are considered “unsafe”. For example, most non-steroid anti-inflammatory agents are contraindicated and the pain must be mainly relieved by the use of opiates. The only available means of etiologic treatment aim at decreasing ALA-S activity. High carbohydrate intake –either per os or through intravenous administration of hypertonic (20%) glucose solutions– is recommended, taking advantage of the aforementioned glucose effect. Administration of heme, direct inhibitor of hepatic ALA-S, is the other therapeutic approach. Several heme formulations have proved problematic until hemin arginate was introduced. This is considerably effective –if administered early– and with minor side-effects, but it is not always available due to high cost and limited use.[6]

Prevention of acute attacks is mainly achieved by avoidance of triggering factors. A well-balanced diet with carbohydrates covering 60-70 % of total calories is indicated. Gonadotropin-releasing hormone analogues can prove useful in women by preventing monthly recurrent attacks related to their menstrual cycle. A prophylactic intravenous heminarginate administration (1-2 weekly) has proved useful in some patients that

could not otherwise behelped.[1] However, all these measures have often been difficult to apply or ineffective. Furthermore, some patients with repeated attacks of pain are prone to opiate dependence.

The introduction of small interfering RNA molecules (siRNAs) seems to be a revolutionary innovation in modern therapeutics. siRNA is a small (20-30 nucleotides) noncoding-synthetic double-stranded RNA designed to specifically target a particular mRNA. Its binding results in mRNA cleavage and degradation. So, siRNAs have currently been introduced in clinical practice in order to selectively target and suppress disease-causing genes. Current investigation focuses on several applications of them in cardiovascular diseases, neurological disorders, or malignancies.[7]

It is interesting that the whole story originated from an attempt to change the color of a common flower, petunia. Napoli et al reported in 1990 that they attempted to overexpress an enzyme responsible for the production of anthocyanin, the pigment of violet petunias by introducing a chimeric gene. Despite their expectations, the introduced gene created a block in anthocyanin biosynthesis.[8] That was the first indication that introduction of a nucleic acid in cells could block protein synthesis. In 1998 Fire and Mello reported that introduction of a double-stranded RNA was substantially more effective at producing interference in the nematode Caenorhabditis elegans than was either strand individually.[9] This phenomenon was called RNA inhibition and offered them a Nobel prize in 2006.

A new agent for the prevention of porphyric attacks has recently been approved by the U.S. Food and Drug Administration (2019) and the European Medicines Agency (2020) under the generic name of givosiran. Givosiran is an siRNA molecule that can selectively bind to the m-RNA for ALA-S. It enters the hepatocytes by binding to the acialglycoprotein receptor of the cell membrane. Entering the cell, it is directed to the ribosomes where it rejects its complementary strand and binds to a molecule of the ALA-S mRNA. This binding resultsin degradation of the latter. Through this mechanism, ALA-S production is considerably decreased.

A phase 1 clinical trial showed that patients with acute porphyria receiving givosiran had a sustainable decrease of ALA-S mRNA and urinary ALA and PBG to near normal levels.[10] Subsequently, a double-blind phase 3 clinical trial (ENVISION) was performed. Givosiran (2.5 mg/kg b.w.) or placebo was subcutaneously administered in 94 symptomatic patients with acute hepatic porphyria once per month for 6 months. The mean annualized attack rate was 74% lower in the givosiran than in the placebo group (3.2 versus 12.5, $p<0.001$). Patients receiving givosiran also had significantly lower levels of urinary ALA and PBG, fewer days of hemin use, and better pain daily scores if suffering of chronic neuropathic pain. After completion of the 6-months period, the patients in the placebo group also passed to givosiran and had similar responses concerning urinary ALA and PBG and attack rate. The effect remained constant with the passing of time. The safety profile of givosiran was good enough with minor and usually reversible elevations of serum creatinine and aminotransferases as well as mild or moderate injection-site reactions. Hepatic heme content or heme-dependent enzyme activities were not strongly affected by the administration of givosiran.[11]

So, intervention with a specific siRNA molecule seems to be an effective way to prevent attacks in patients with acute hepatic porphyrias. This treatment is expected to greatly improve the quality of life of these patients and also save the cost for their hospitalization or drug treatment. However, there are some questions that remain unanswered. Will the effect of givosiran persist over more prolonged time-periods? May its prolonged use and ALA-S inhibition be accompanied by other side-effects? And more, important: may this treatment also ameliorate the chronic complications of porphyria, such as renal damage, psychiatric disorders or liver cancer? These are questions that may only be answered through the long-term use of givosiran or similar drugs.

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