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Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias

(δ-aminolevulinate synthase/derepression/induction/coproporphyria)

C. J. WATSON, C. A. PIERACH, IRENE BOSSENMAIER, AND RUTH CARDINAL

University of Minnesota Medical Research Unit, Northwestern Hospital, Minneapolis, Minnesota 55407

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ABSTRACT **There is compelling, indirect evidence of hepatic heme deficiency due primarily to the respective genetic errors of the three inducible hepatic porphyrias, acute intermittent porphyria, porphyria variegata, and hereditary coproporphyria. The induction is enhanced by exogenous inducers such as barbiturate, estrogens, and other "porphyrogenic" chemicals and factors, including glucose deprivation. The newer knowledge of the induction of δ-aminolevulinic acid synthetase [δ-aminolevulinate synthase; succinyl-CoA:glycine *C*-succinyltransferase (decarboxylating), EC 2.3.1.37] in relation to inadequate heme, and repression by heme, stimulated early trials of hematin infusions to overcome the acute relapse in the foregoing inducible porphyrias. Recently this experience has been considerably expanded, 143 infusions of hematin having been given in 22 cases. Studies of the effect on the serum concentrations of δ-aminolevulinic acid and porphobilinogen have shown a highly significant decline, often to 0, especially of δ-aminolevulinic acid. A distinct relationship to the clinical severity of the attack has been evident in the frequency and magnitude of decline of serum δ-aminolevulinic acid and porphobilinogen. This was regularly associated with objective clinical improvement.**

The present preliminary report will consider briefly some basic and associated clinical aspects of the remission due to hematin, in cases of "inducible" hepatic porphyria. In a later paper this effect will be considered in greater detail (1).

Those forms of hepatic porphyria in which various inducing factors are liable to precipitate a life-threatening acute neurologic relapse are appropriately grouped under the term *inducible* (1). This relates to δ-aminolevulinic acid synthetase [ALA-synthetase; δ-aminolevulinate synthase; succinyl-CoA: glycine *C*-succinyltransferase (decarboxylating), EC 2.3.1.37], the rate-limiting enzyme of porphyrin and heme biosynthesis (2–6). The term *inducible* embraces the three autosomal dominant genetic errors, acute intermittent porphyria (AIP), porphyria variegata (PV), and hereditary hepatic coproporphyria (HC), but it does not include hepatic porphyria cutanea tarda, in which evidence of induction is slight or lacking (7, 8), as well as the neurologic symptoms of the acute attack in the inducible forms and the prominent excesses of porphyrin precursors, ALA and porphobilinogen (PBG) in liver, urine, and blood serum, increases of which are usually observed at least in the early symptomatic stage of the porphyric relapse. The inducible forms are characterized by derepression or impaired feedback regulation and secondary induction of hepatic ALA-synthetase (6) (see above). There is reason to believe that this is related to hepatic heme deficiency incident to the respective genetic (partial) lack of uroporphyrinogen synthetase in AIP (6), heme synthetase in PV (7), and coproporphyrinogenase in HC (9). Any one of these traits constitutes a partial block in heme synthesis, responsible, as already mentioned, for the negative feedback induction of ALA-synthetase (2–6). In production of the acute attack, this is usually augmented by an exogenous factor.

The repressive or inhibitory feedback effect of heme on ALA-synthetase induction (5, 6) led to a trial, by Bonkowsky *et al.* (10), of hematin infusions in AIP. The subject also suffered from "malignant" primary hypertension with renal insufficiency and uremia leading to early demise, but probably unrelated in any direct fashion to the porphyric relapse. This first trial of hematin was of particular significance in revealing its striking effect on the serum porphyrin precursor levels. (The patient was almost totally anuric, hence urinary studies were minimal.) Each infusion was followed by a highly significant decline of serum ALA and PBG. This was soon confirmed in several case studies (11–14) reported from this laboratory, the decline being regularly associated with rapid clinical remission. Nevertheless, in porphyria roster no. 430 (P430), one of PV, our first patient thus treated (12), hematin was deferred for 3 days pending a possible spontaneous, or glucose-induced improvement (15), but this was not observed, respiratory paralysis and quadriplegia persisting. Tracheostomy and assisted respiration were probably life-saving, but because the patient's status was still highly precarious, hematin was administered in two 250-mg infusions each day, at an interval of 2 days, for a total of 1 g. There was progressive improvement of the respiratory paralysis and slower return of function in the legs, with a permanent left peroneal palsy. Thus, while the result in P430 was not auspicious, it may nevertheless have permitted survival and recovery from a nearly moribund state. This would not be surprising, in the light of subsequent experience in which rapid remissions have been usual.

MATERIALS AND METHODS

The preparation of crystalline hemin and of the hematin solution (12) for infusion is to be described in detail elsewhere (1), also a recent, preferred manner of infusion. The latter is mentioned briefly in the following.

The hematin solution (0.25% Na_2CO_3) adjusted with HCl to pH 7.8–8.0, containing 4 mg/kg of body weight, is infused by hand over 10–12 min, through a plastic tube attached to an inlying 21-G Butterfly® infusion set. The tube is connected with a saline bottle, and when the infusion is complete, the system is flushed with saline prior to removal of the needle. In many infusions this technique has been unaccompanied by vein irritation or phlebitis, such as were encountered at times with the conventional method (12). The number of infusions and interval between has been related roughly to the "severity" class A, B, or C, as will be discussed elsewhere (1). In brief, this depends on type and severity of the dominant symptoms; class A = autonomic and early peripheral neuritic; A_1 = chemically identified porphyria with chronic psychoneurosis of unknown relation to the porphyria (16); class B = autonomic plus severe peripheral neuropathy, often leading to quadriplegia; class C = quadriplegia plus bulbar and/or respiratory paralysis. It is

Abbreviations: ALA, δ-aminolevulinic acid; PBG, porphobilinogen; AIP, acute intermittent porphyria; PV, porphyria variegata; HC, hereditary hepatic coproporphyria; P no., porphyria roster no.

recognized that there may be spontaneous change in untreated cases in either direction, though more often for the worse.

In the present study, hematin has been infused in 22 patients. This includes one of congenital erythropoietic porphyria (8 infusions) (17) and one of porphyria cutanea tarda (7 infusions) (12), neither of which will be considered here other than to underline the lack of adverse reactions, in common with 20 patients having one or another form of inducible porphyria (1): AIP, 13; PV, 6; HC, 1. In these, 128 infusions have been given for 31 attacks.

For 14 attacks in 11 patients in other centers, hematin solution (1, 12), ready for infusion, except for final filtration (0.20 μm) to insure sterility, was provided by air transport to be administered in the treatment of life-threatening attacks. Serial samples of serum were obtained at appropriate times by the attending physician and transmitted to this laboratory for analysis of porphyrin precursors and (in selected cases) porphyrins. Urinary and fecal porphyrin determinations (18) were also carried out whenever possible, to establish the type of porphyria and obtain serial data relating to the question of induction of ALA-synthetase in PV and HC, in both of which the abnormal porphyrin metabolism is often represented largely by proto- and/or coproporphyrin in the feces rather than ALA or PBG in urine or serum, as in AIP.

RESULTS AND DISCUSSION

Of the foregoing 31 attacks in 20 patients with inducible porphyria receiving hematin, prompt and often dramatic recovery was observed in 25 (1). Nevertheless, two of the series died, P469 and P486; both were studied elsewhere and their attending physicians cooperated in providing essential information and samples for analysis. Death in both cases was reported to be due to pulmonary complications of respiratory paralysis and tracheostomy or intubation. The attending physician stated that in P469 respiration had apparently just begun to improve after hematin, when the patient (a chronic alcoholic) was afflicted with staphylococcal pneumonia, and very soon succumbed (1). The second patient, P486, showed distinct improvement following hematin, with improved muscle strength, return of gag reflex, and clearing of sensorium, but prior to hematin and a sequel to respiratory paralysis, had developed a "shock" lung, bronchopleural fistula, and cardiac complications, jointly regarded as the cause of death.

In an earlier case (P453), in which the patient was considered to be nearly moribund with respiratory paralysis and quadriplegia, there was a very gratifying recovery following hematin. This case has been reported in more detail elsewhere (13).

The series includes P471 (PV), a woman, age 36, who had two attacks at an interval of 6 months, the first of which followed a small bowel bypass for obesity, with a resultant weight loss of 207 pounds over a 2-year period, in relation to which glucose deprivation was believed highly adverse for the porphyria (15). She was given Premarin®*, Valium®, and Meperidine® for abdominal pain, and developed a severe attack with respiratory difficulty (partial respiratory paralysis). Following hematin infusions totalling 864 mg in 4 days, she made a good recovery, and after a time in a nursing home, returned to her home. Then, however, "boyfriend problems" produced depression and she went on a fast, following which she had an acute porphyric relapse believed due to the low glucose intake, and received 1200 mg of hematin in 6 days. The serum ALA, 32 μg/100 ml before hematin, fell to 0, likewise, serum PBG from 131 μg/100 ml to 0. This was accompanied by rapid, outspoken improvement in the autonomic and respiratory status and in the feeling of well being. The quadriparesis improved much more slowly, as is usual (1).

The possibility must be entertained that the repressive effect of hematin, in acute porphyric relapse, may be interfered with, in some measure, by continued exogenous inducer activity, as for example, Dilantin® given for porphyria-related seizures. This will be considered elsewhere in more detail (1). At least equally important is the avoidance of delay in administering hematin for the acute relapse of an inducible porphyria. Early infusions of hematin have in all likelihood avoided or minimized respiratory paralysis and the pneumonia which may fatally complicate its treatment, as in cases P469 and P486 of the present series. In both, these complications were already well developed when hematin was commenced, reemphasizing the need for prompt administration. The corollary of this is the early testing of the urine for PBG, invariably present in the acute attack of AIP, but in PV or HC often disappearing even when the patient is still symptomatic. The modified Watson–Schwartz test (20) for PBG is widely used and generally reliable. The Hoesch test has recently been advocated (21) and is presently being studied as to relative sensitivity and specificity.

The concept of hepatic heme deficiency in the inducible porphyrias is not based on actual measurement of heme, but rather on the large amount of porphyrin precursor in the liver, serum, and excreta, together with the evidence cited earlier that this is related to derepression or negative feedback induction of ALA-synthetase because of heme deficiency (6), and also the decisive decline of the serum ALA and PBG after hematin (see below). In this regard, the early findings by Prunty (22) and Tschudy *et al.* (4) of large amounts of PBG and ALA-synthetase, respectively, in the livers of patients with fatal AIP, are highly significant.

Determination of the serum porphyrin precursors by Miyagi *et al.* (23) has aided materially in estimating the degree of overproduction and the highly significant decline associated with hematin administration, regularly correlated in our experience, with clinical improvement. The serum PBG, prehematin, generally exceeds ALA in AIP and in PV, in relapse. After hematin the serum ALA falls consistently, usually to 0. The serum PBG also declines in all instances, though often to a lesser extent than the ALA, which may be related to conversion of ALA to PBG in liver or blood (dehydrase activity). These data reveal the marked degree to which induction of ALA-synthetase and resultant increase of ALA and PBG are repressed by hematin. We have observed oscillations of the serum ALA after hematin (13), reminiscent of those noted in experimental porphyria (24).

A definite though only partial correlation was evident between the severity of the attack (A, B, or C, as above) and the degree to which ALA and PBG declined, after hematin. In all of the 10 attacks of class A severity, the serum ALA fell to 0; this was true in but two of five in class B, and but one of two in class C. The decline of the serum PBG after hematin was less consistent, nevertheless significant, four of 10 in class A falling to 0, while of the seven in classes B and C combined, for which data were available, but two declined to 0. In 10 of 17 there was greater than an 80% decline of the sum of ALA and PBG after hematin.

The apparent correlation of severity of the attack, with heme deficiency, as judged by the increase of the porphyrin precursors (ALA and PBG) and precursor porphyrins (uroporphyrinogen, coproporphyrinogen, and protoporphyrin) in evidence is quite in accord with the prompt remission following hematin infusions. It is interesting that this also appears to apply in the two forms of inducible porphyria (PV and HC) in which

* Potentially porphyrogenic, in common with other estrogenic compounds (19).

the precursor porphyrins are found in large excess in the feces in association with smaller, or even negligible, amounts of porphyrin precursors in the urine, the latter often disappearing entirely in remission. In a recent case of HC (P485) it was difficult, if not impossible, to classify the patient in terms of relapse or remission. She had a longstanding "emesis nervosa" of questionable relationship to the coproporphyria, and without other clinical manifestations of porphyria. The urine contained only insignificant amounts of ALA and PBG, but both urine and feces exhibited large excesses of coproporphyrin, the 24-hr urine containing 4068 μg/day, normally <280; the feces 97,000 μg/100 g, 4418 μg/g dry weight, normally <32.7. The serum coproporphyrin was 2.3 μg/100 ml; ALA and PBG both recorded as 0. Infusions of hematin in this case were followed by a remarkable decline of the urine coproporphyrin to 161 μg/day, well within the normal range; the fecal coproporphyrin fell to 145 μg/g dry weight. The prominent diminution of both urinary and fecal coproporphyrin after hematin is believed to indicate that a highly significant induction of hepatic ALA-synthetase gave rise to the greatly excessive coproporphyrin formation, which was repressed by the hematin. In this connection, Elder and associates (9), in a recent paper, note that uroporphyrinogen-1-synthetase becomes rate limiting in hepatic heme synthesis in HC, "with consequent accumulation of ALA and PBG." This is of special interest in respect to the various observations in P485, especially the negligible serum and urine porphyrin precursors, and the remarkably large amounts of coproporphyrin in feces and urine. Elder *et al.* (9) believe that the liver in HC may provide as much as a "30-fold increase in the rate of hepatic haem synthesis . . . provided saturating substrate concentrations can be achieved." At the same time, "the acute attacks . . . appear to be the clinical counterpart of limitation of the rate of haem synthesis by uroporphyrinogen-1-synthase." Whether a theoretical 30-fold increase would be adequate is unknown. Variability in the rate of heme destruction and the induction of hepatic heme oxygenase (25) must also be considered, as well as the microsomal oxidative degradation of heme (26).

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