ENZYME REPLACEMENT THERAPY IN PORPHYRIAS IV. FIRST SUCCESSFUL HUMAN CLINICAL TRIAL OF δ-AMINOLEVULINATE DEHYDRATASE-LOADED ERYTHROCYTE GHOSTS

Alicia M. Del C. Batle1, Norma L. Bustos1, Ana Maria Stella1, Eva A. Wider1, Honorina A. Conti1* and Armando Mendez1*

1Centro de Investigaciones sobre Porfirias y Porfirias, CIPYP (CONICET y FCE y N. University of B. Aires), Ciudad Universitaria, Pabellon II, 1428 Buenos Aires, Argentine
2Unidad B-4 de Clinica Médica, Hospital Ramos Mejia, Gual. Uzquiza 609, 1221 Buenos Aires, Argentine

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Abstract—1. A patient with chronic lead intoxication was treated with only one course of highly purified human blood aminolaevulinate dehydratase entrapped in autologous erythrocyte ghosts given intravenously.
2. No untoward effects were observed during or after infusion.
3. An immediate increase in the patient's erythrocyte dehydratase activity was detected 1 hr after enzyme administration, reaching its maximum and nearly normal level 2 days later, values remained unchanged for a week, to slowly diminish after 2 weeks of initiated the treatment, and finally recovered activity was kept practically levelled off for weeks.
4. This novel therapeutic trial produced complete improvement both clinical and biochemical, showing that enzyme infusion has been beneficial and can be safely and successfully used in the treatment of human lead intoxication.

INTRODUCTION

Enzyme replacement therapy of genetically inherited disorders has received increasing attention (Ryman, 1977). We have extended its possibilities to the field of porphyrias, which are in fact both hereditary and acquired disturbances in porphyrin metabolism. In each type of porphyria a specific and single enzyme is partially defective, leading to an abnormal and characteristic biochemical pattern of accumulation and excretion of porphyrins and/or precursors. Therefore, we became enthusiastic in the application of enzyme therapy in these diseases, an approach not yet tried before (Bustos et al., 1980, 1983a; Espinola et al., 1983).

In order to successfully apply enzyme replacement therapy, some criteria must be fulfilled: (i) a suitable carrier for the enzyme, which protects both the enzyme from degradation and inactivation and the host from unwanted pharmacological and immunological effects, should be developed; (ii) a source of enzyme should be selected, this aspect is of major importance, and, when planning to be used in human therapy, it should be of human origin; (iii) the preparation of the enzyme should be of the highest purity possible and very stable; (iv) the enzyme should be delivered to the tissue in which it is to function; (v) the enzyme should interact with its substrate long enough to render clinical benefits.

In selecting the porphyria to start trying enzyme replacement therapy, we chose lead intoxication, because of many considerations. It is a disorder which occurs with high frequency, consequently it would be easy to find suitable test subjects and also the number of people benefiting would be important. It is one of the most advantageous experimental animal models. An important biochemical abnormality in lead poisoning is an increased concentration of δ-aminolaevulinc acid (ALA), very easily detected in urine, as a consequence of a significant inhibition of ALA-dehydratase (ALAD) by the metal; measurement of erythrocyte ALA-D activity is the most sensitive indicator of lead exposure.

Therefore, a procedure for obtaining a highly purified and stable preparation of ALA-D from human blood has been developed (Bustos et al., 1980).

The potential use of erythrocytes as carriers for ALA-D was investigated and we found that resealed erythrocyte ghosts can function as such, entrapping the enzyme with high yield and activity and delivering it to the desired target sites in the very cells which produce the metabolic disruption (Bustos et al., 1983a).

Results on animal models, showed that it was possible to correct both in vitro (Bustos et al., 1983a) and in vivo (Bustos et al., 1983b) the activity of defective erythrocytes by incorporating exogenous human enzyme, indicating that the cell ghosts loaded with human ALA-D could be useful for treatment of human lead intoxication.

We now report clinical and laboratory findings in a patient with chronic lead intoxication whom we have treated with human blood ALA-D entrapped in his own red cell ghosts.

MATERIALS AND METHODS

Blood samples
Normal human blood (3 l) for enzyme purification was obtained from the Blood Bank of Ramos Mejia Hospital, Buenos Aires. Patient's blood (500 ml) was withdrawn directly into a standard blood bag containing heparin.

Purification of human blood ALA-D
This was carried out following the procedure described by Bustos et al. (1980). ALA-D activity was assayed according to Battle et al. (1965) along the steps of the purification procedure or to Bustos et al. (1983a) when measured in both intact erythrocytes and ghosts. One unit of ALA-D is defined as the amount of enzyme catalysing the formation of 1 nmol PBG/h; activity will be expressed either as Units/mg protein (U/mg) or Units/ml of packed red cells (U/ml). Porphyrias and PBG were measured by the method of Rimington (1971) and ALA by that of Mauzerall and Granick (1956). Blood lead was determined by flameless atomic absorption spectrophotometry following a procedure similar to that described by Kesten et al. (1980).

Preparation of autologous erythrocyte ghosts containing ALA-D
Red blood cell ghosts containing ALA-D (RBCg(ALA-D)) were prepared from 500 ml of patient's own blood, following the technique described by Bustos et al. (1982), yielding 100 ml of ghosts, which were suspended in equal volume of isotonic saline (activity 2.47 U/mg or 128.4 U/ml). Porphyrins and PBG were measured by the method of Rimington (1971) and ALA by that of Mauzerall and Granick (1956). Blood lead was determined by flameless atomic absorption spectrophotometry following a procedure similar to that described by Kesten et al. (1980).

RESULTS AND DISCUSSION

Clinico-biochemical observations
From complete clinical examinations and analysis, acute lead intoxication was suspected, the diagnosis was confirmed on the basis of excess urinary excretion of ALA and porphyrins, mainly Co-proporphyrin (PBG was also slightly increased at admission), significantly decreased activity of erythrocyte ALA-D and augmented levels of Pb in blood (Table 1, 25/1/82). Typical basophilic stippling of the red blood cells, moderate anaemia, reticulocytosis and the lead line on the gingival were also observed. Peripheral neuropathy has been documented by means of nerve conduction studies and electromyography. His abdominal colic was treated with dextrose solution (10%,) and calcium gluconate, leaving the hospital 2 weeks later. Sickness, nausea, vomiting, abdominal colics, general sense of malaise, myalgia, insomnia, including sexual impotence, related to his polyneuritis (already reported at the first admission) continued; therefore the patient was hospitalized again for chelation therapy on February 15th, 1982. Laboratory data are shown in Table 1. A total of 20 doses of BAL (3 mg/kg, i.m.), and i.v. infusion over 4 hr, of Ca- Na,FDTA (two 20% ampoules, in 500 ml of dextrose 10%, daily) during 8 days, were given.

The patient was discharged immediately after treatment without major changes in the urinary levels of precursors, Pb in blood or the activity of ALA-D, as can be seen in data corresponding to 5/4/82 and 26/4/82 in Table 1.

Although lead intoxication is an acquired disorder of porphyrin metabolism, a family study of some consanguineous relatives of the patient was carried out on the day of his second admission (Table 2). It was very interesting to find that his wife and three children (all of them living in the same house), although not showing any clinical symptoms, have low levels of ALA-D; particularly his two sons (cases 3 and 5), who helped him with the plumbing. The eldest son also has Pb levels above normal and both urinary precursors and porphyrins up to the highest normal limit. On the other hand, two sisters (cases 6 and 7) who live apart, showed no abnormality at all.

<table>
<thead>
<tr>
<th>Date</th>
<th>ALA (mg/24 hr)</th>
<th>PBG (mg/24 hr)</th>
<th>Urinary porphyrins (µg/24 hr)</th>
<th>Fecal porphyrins (µg/g dry wt)</th>
<th>ALA-D (U/ml)</th>
<th>Pb (µg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/1/82</td>
<td>19.98</td>
<td>4.26</td>
<td>196.8</td>
<td>1937.4</td>
<td>44</td>
<td>71</td>
</tr>
<tr>
<td>15/2/82</td>
<td>9.76</td>
<td>3.27</td>
<td>197.2</td>
<td>1019.6</td>
<td>27</td>
<td>105</td>
</tr>
<tr>
<td>5/4/82</td>
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<td>2.27</td>
<td>130.1</td>
<td>752.8</td>
<td>58</td>
<td>123</td>
</tr>
<tr>
<td>26/4/82</td>
<td>9.41</td>
<td>2.99</td>
<td>23.9</td>
<td>330.1</td>
<td>83</td>
<td>125</td>
</tr>
<tr>
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<td>2–4</td>
<td>1–2</td>
<td></td>
<td>20–250</td>
<td>656±126</td>
<td>0–35</td>
</tr>
</tbody>
</table>

Determinations were carried out as described in "Materials and Methods"
Enzyme replacement therapy in porphyrias—IV

Application of enzyme replacement therapy

Quelation therapy in this patient did not produce major benefit either clinically or biochemically. At the beginning of December 1982 the laboratory data were much the same as those of previous studies; therefore we decided to apply enzyme replacement therapy by using ALA-D loaded RBC ghosts.

In spite of a number of attempts, enzyme therapy has not as yet been proved to be of practical use in the treatment of any human disease. However, we did consider that all the conditions were already established to be successful this time. As already stated, our preliminary studies in animals, showed that administration of ALA-D entrapped in RBC ghosts to lead intoxicated animals was absolutely safe and could overcome the biochemical defect, restoring the greatly reduced ALA-D activity to normal levels (Bustos et al., 1983b).

The patient was carefully informed about the new therapeutical trial and gave unequivocal consent. On December 6th, the patient was then treated with 100 ml of autologous RBCg loaded with 25,600 units of ALA-D. He was permanently controlled for the first 24 h, enzyme infusion showed no effect on blood pressure, respiration rate, pulse, body temperature, or any other vital signs. No abnormalities in renal or liver function tests were observed either and not any other subjective symptoms were associated with the carrier-assisted delivery of ALA-D.

Samples of blood and urine were collected at different times during the first day, then blood, urine and feces, daily for 11 days and then every 34 days. As is shown in Fig. 1, significant increase in his ALA-D activity was seen after 1 hr, a peak at 2 hr was observed, followed by a 7-10x decrease at 4 hr, and then a constant recovery of activity at 16, 24 hr, reaching its maximum 2 days after infusion, which was maintained for the next week. At day 10 the levels began to slowly diminish up to day 14, when he was discharged, from then onwards the activity of ALA-D remained practically leveled off for the following 3 weeks.

We should note and emphasize here that, results obtained with the patient, exactly reproduced the animal response when using the RBC ghosts mode of ALA-D administration to lead intoxicated rats and mice, a peak of activity after 2 hr of infusion was constantly observed (Bustos et al., 1983b).

It was also found that the urinary excretion of precursors and porphyrins returned rapidly to normal and the level of Pb in blood was reduced by about 45% (Figure 1).

Although it is often difficult to appraise subjective changes, rapid improvement was observed in our patient's activity status and in his feeling of well-being, during treatment. We have not detected any untoward side-effects, neither any abnormality in coagulation screening or liver function tests during or after infusion. He presented no symptoms and all clinical and biochemical parameters were normalized.

It is interesting to recall that since his first admission the patient complained of sexual impotence, after receiving ALA-D loaded RBC ghosts he said he has completely recovered his sexual capacity in full. We know that oral exposure to very low doses of Pb
Fig. 1. Effect of enzyme replacement therapy with ALA-D loaded autologous erythrocyte ghosts in a patient with chronic lead intoxication on ALA-D levels (△), urinary ALA (○), urinary PBG (●), urinary porphyrins (▼) and Pb in blood (▼). The patient received only one course of enzyme at 0 time. Experimental details are given in the text.

(2 μ/kg body wt) causes damage to spermatogenesis and to RNA synthesis in the cell division phase (Egarova et al., 1966) and andrological disturbances with sexual disfunction have been reported in men exposed to leaded gasoline (Neshkov, 1971) and inorganic lead (Lanceranjean et al., 1975). On the other hand, removal of polyvalent metals, including lead, by means of quelation, drastically increases the life span of spermatozoa (Bjorksten, 1968). The recovery of sexual function in our patient is another clear sign of the benefit produced by the therapy and we might speculate that the burden of ALA-D given in the RBC ghosts has not only metabolized the excess ALA and restored the enzyme levels to nearly normal, but it is also possible that part of the enzyme has been acting as a kind of quelate, sequestrating the circulating Pb.

It is very likely that infusion of larger amounts of enzyme would be even more effective in the management of the disease, probably magnifying the effects observed here; for instance further decreasing the levels of Pb in blood.

CONCLUSIONS

It has been stated that enzyme replacement therapy is a promising therapeutic approach which nevertheless had not as yet been achieved (Beutler, 1981). Erythrocyte ghosts have many of the attributes of the ideal carrier for enzymes (Ihler, 1979), one very important attribute is that the patient's own blood may be used. However, so far, the only report of its application in a human clinical trial has been the unsuccessful attempt to treat Gaucher's disease by administration of glucocerebrosidase encapsulated in human red cells coated with gammaglobulin (Beutler et al., 1977). In an interesting paper the authors demonstrated the safety of the treatment, although the results obtained had been of doubtful benefit, in a patient with far-advanced disease, who finally died.

Our preliminary studies in animal models (Bustos et al., 1983b) were very encouraging indeed and prompted us to initiate replacement therapy with ALA-D in a chronic case of lead intoxication, because, obviously, clinical efficacy must only be evaluated in clinical trials.

As far as we know this is the first successful human clinical application of the use of an enzyme encapsulated in erythrocyte ghosts for treating a disease. The present studies have been confined to the use of autologous red cells, however donor cells can also be used for enzyme entrapment.

The current treatment of lead intoxication involves chelation therapy, very often with agents such as BAL, EDTA or penicillamine, which are very hazardous, so care should be taken on the possible toxic effects of the medication. The treatment proposed here is relatively free of side effects.
Our results indicate that a number of lead intoxicated patients could benefit from this new form of therapy.

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REFERENCES


