ISSN 004-5772

JOURNAL OF ASSOCIATION OF PHYSICIANS OF INDIA

VOL 41 Editor: P. J. MEHTA JUNE 1993

ORIGINAL ARTICLE

AUTOANTIBODIES IN THALASSAEMIA MAJOR : RELATIONSHIP WITH ORAL IRON CHELATOR L1

J Mehta*, A Chablani**, R Reporter***, S Singhal*, BC Mehta****

ABSTRACT

Ninety patients with thalassaemia major were investigated for the occurrence of antinuclear antibodies (ANA), and those with ANA were tested for antibodies to histones (AHA). ANA were detected in 7 of 27 thalassemics on oral iron chelator L1, and in 2 of 63 thalassaemics not on L1 ($p < 0.01$). AHA were seen in 4 of 7 thalassemics receiving L1 with positive ANA, and in none of the 2 not receiving L1 ($p < 0.03$). Joint pains were seen in patients receiving L1, but in none of the patients not receiving L1. There was no correlation between hepatitis B or HIV positivity and presence of ANA or joint pains. While some amount of background ANA-positivity was found in patients with thalassaemia major, it was significantly more in patients receiving L1. Laboratory evidence of drug-induced lupus-like reaction was seen only in patients who received L1. In view of serious concerns about the safety of L1 and wide variations in the incidence and severity of adverse reactions reported by different sources, an urgent regulatory audit of all trial centres is essential.

INTRODUCTION

Long-term iron chelation therapy with desferrioxamine in patients with transfusion-dependent thalassaemia is well-established, generally safe and reduces or prevents iron-induced organ dysfunction.[1] Desferrioxamine is the standard against which the risks and benefits of any new form of chelation therapy including oral iron chelators should be measured.[2]

Of the large number of drugs that have been evaluated as potential oral iron chelators, the hydroxypyridinone family has been the most extensively studied.[3] 1,2-dimethyl-3-hydroxypyridin-4-one (L1 or CP20) has been the most widely studied oral chelator. While some investigators have found it to be a safe drug in animal studies,[4,5] others have encountered substantial animal toxicity such as potentiation of the action of barbiturates in rats,[6] increased salivation, severe sweating, muscular spasms, and hyperactivity.[3,7] Agranulocytosis and thrombocytopoenia developed in one L1 recipient with Blackfan-Diamond syndrome.[8] Agranulocytosis developed in 3 of 34 patients receiving L1 in London.[9]

We have described a case of fatal L1-induced systemic lupus erythematosus.[10] The reports of occurrence of autoantibodies in thalassaemics receiving L1 have been conflicting,[11-20] and questions have been raised about a backgound occurrence of antinuclaer antibodies (ANA) in patients with thalassaemia major.[13,20] Antibodies to nuclear histones (AHA), which are supposed to be relatively specific for drug-induced lupus,[21] have been described in patients with thalassaemia major not receiving L1.[17]

The present study was undertaken to determine the frequency of occurrence of ANA in thalassaemics irrespective of the kind of chelation therapy they were receiving, and to see if the presence of ANA was drug-related by determining AHA. The data have been presented in abstract form.[22]

MATERIAL AND METHODS

All the thalassaemics attending two major blood transfusion centres (Nanavati Hospital Blood Bank and Tata Blood Bank, JJ Hospital) in Bombay were investigated. Detailed inquiry was made about arthralgia and the nature of chelation therapy. Information about the HBsAg (ELISA) and HIV (ELISA with Western Blot confirmation of positive results) status was obtained from recent records.

ANA were estimated in all patients by immunofluorescence using HEp-2 cells as substrate. All the samples found to be positive for ANA were further studied for antibodies to double-stranded DNA (AdsDNA) by immunofluorescence using *Crithidia luciliae* as substrate, and for AHA by ELISA (Sigma).

RESULTS

A total of 90 thalassaemics were investigated, of whom 27 were receiving L1 and 63 were not receiving L1. Of the latter, 21 were on regular desferrioxamine. The dose of L1 was 500 mg to 4 g per day.

Eight patients in the L1 group had arthralgia while on L1, and one had joint swellings. One of the patients had arthralgia even before starting L1 and this was aggravated after starting L1. In this

*Blood Research Centre, Vivina Bldg 3A, SV Road, Bombay 400 058; Leukaemia Unit, Royal Marsden Hospital, Sutton, Surrey. **Immunopathological Diagnostic Centre, 14 Parekh Mahal, LJ Road, Bombay 400 016; ***Tata Blood Bank, JJ Hospital, ****Nanavati Hospital, SV Road, Vile Parle, Bombay 400 054.
Received: 17.2.1993; Revised: 10.5.1993; Accepted: 21.5.1993

patient and in two others, L1 had to be discontinued due to the severity of the pain. L1 was restarted in one of the latter, resulting in severe arthralgia necessitating L1 discontinuation once again. L1 was restarted in the latter two at low dose (500-1000 mg per day) without recurrence of arthralgia. Joint pain was not present in any of the patients not receiving L1. Seven patients were HBsAg-positive and five were HIV-positive. None of these 12 had arthralgia.

The pattern of autoantibodies detected is shown in Table 1. AdsDNA were not detected in any of the nine patients with positive ANA. The ANA titre was 1:40 and 1:80 in the two patients not receiving L1, and ranged from 1:80 to 1:160 in the seven patients receiving L1. None of the HBsAg-positive patients had a positive ANA, while one of the HIV-positive patients had a positive ANA but negative AHA.

Table 1 : Immunological investigtions in 90 thalassaemics

	ANA-positive	AHA-positive
Thalassaemics on L_1 (n = 27)	7 (25.9%)	4 (14.8%)
Thalassaemics not on L_1 (n = 63)	2 (3.3%)	0 (0%)
P	< 0.01	< 0.03

Five months after discontinuation of L1 on our advice, one of the patients who had positive ANA and AHA became negative for both. Four months after discontinuation of L1, one patient who had positive ANA and a negative AHA became negative for ANA.

DISCUSSION

Bartlett et al reported the development of transient arhralgia in 5 of 13 patients on L1;[11] two of whom became seropositive for RA. One patient developed ANA (1:40) during L1 therapy, and the other showed no change in a pre-L1 positive ANA (1:20). The remaining three were ANA-negative.

There has been much conflict and inconsistency in the way all three groups involved with human trials of L1 have addressed the issue of autoimmunity as a result of the drug. The Canadian group investigating L1 did not describe any immunological abnormalities in their detailed report,[12] but later reported positive ANA in 5 of 12 thalassaemics with negative anti-dsDNA and AHA before starting L1[13] in response to our report of L1-induced SLE.[10] No mention was made of the subsequent evolution of these abnormalities during the course of therapy with L1.

Al-Refaie et al reported presence of ANA in 2 of 12 patients prior to starting L1, and in 4 of 12 after conclusion of the trial.[14] However, no mention was made of AHA despite the fact that the presence of AHA is supposed to be specific for drug-induced lupus. This was an omission which we had to point out in view of serious concern about L1-induced SLE.[23]

The most striking contradictions have come from the Bombay-based group investigating L1. Three of 24 Indian patients with thalassemia major on L1 developed transient musculoskeletal pain and arthralgia.[15] All three were reportedly negative for ANA, AdsDNA, and RA factor. The phase II L1 trial report on 52 thalassaemics in India[16] showed GI tract symptoms in 13.5% and arthralgia in 21.1%. In the same report, Agarwal et al found no "significant toxicity" on "extensive clinical and laboratory monitoring" of several organ systems and functions including the immune system.[16]

However, our investigation of two patients from Agarwal et al's cohort disclosed evidence of drug-induced SLE in August 1990. One had severe constitutional symptoms of SLE and died two months after the diagnosis of SLE with active disease despite aggressive therapy with corticosteroids.[10] The other patient was asymptomatic, and only had the triad of investigations considered suggestive of drug-induced lupus: positive ANA, negative AdsDNA, and positive AHA.[21]

Agarwal et al did report presence of ANA[17-19] and AHA[17] after we described L1-induced lupus.[17] The question of immunologic alterations observed in Indian patients receiving L1 was not adequately addressed in any of these. In a detailed report on the efficacy of the drug,[18] Agarwal et al made no mention of the patient who had died due to L1-induced SLE.[10] In their final trial report.[19] Agarwal et al made no mention of AHA at all wich could have shed some light on the drug-induced lupus controversy.

The dat presented by Agarwal et al suggests that the incidence of ANA-positivity is similar in thalassaemics receiving L1 and those not receiving L1,[17-19] and that AHA may be seen in patients not on L1.[17] The latter would conflict with the accepted belief that AHA are specific for drug-induced SLE.[21] Our work presented in this paper with some patients on the L1 trial and other thalassaemics from the same socioeconomic and ethnic background shows that while some amount of background ANA positivity occurs in thalassaemics not taking L1, there is a significantly higher incidence of ANA positivity in thalassaemics taking L1. We could not detect any AHA in patients not taking L1.

Berdoukas hypothesized that high frequency of hepatitis B positive blood administrations at transfusion centres in Bombay and the resultant chronic hepatitis may account for systemic arthropathy in Indian thalassaemics receiving L1.[20] The present study finds no correlation between the HBsAg or HIV status of the patient and joint pains or autoantibodies.

When the first serious doubts about the use of L1 in

human subjects were raised by us with a call for halting human trials pending detailed immunological testing of patients,[10] Berdoukas felt that our concerns were unsupported and that the value of immunological investigations was "unclear".[20] Berdoukas et al now feel that there is "serious doubt" about the safety of this agent and that further development of this compound is "not justified".[24] This is based upon extensive animal studies which show that the pattern of toxicities of L1 is similar to that of a cytotoxic agent with teratogenicity, mutagenicity and dose-dependent myelosuppression.[24]

In light of the animal and human toxicity data reported with L1 so far, it is surprising that the International Study Group on Oral Iron Chelation (ISGOIC) feels that discontinuation of further development of L1 is "premature".[25] Considering the obvious reported inconsistencies in various types of adverse reactions and conflicts of interest, it is possible that L1 is in fact more toxic in human subjects than has been reported so far. Before the appeal of the ISGOIC to carry out further trials with L1 is considered, in the interest of patients, all the three major human trials of L1 in thalassaemia major (India, UK and Canada) need to be audited urgently by the relevant regulatory drug authorities and independent investigators to see if any additional important aspects need to be highlighted.

REFERENCES

1. Hoffbrand AV, Wonke B. Results of long-term subcutaneous desferrioxamine therapy. *Bailliere's Clin Haematol* 1989; 2 : 345-362.
2. Cohen A. Current status of iron chelation therapy with desferrioxmine. *Semin Hematol* 1990; 27 : 86-90.
3. Porter JB, Hider RC, Huehns ER. Update on the hydroxypyridinone oral iron chelating agents. *Semin Hematol* 1990; 27 : 95-100.
4. Kontoghiorghes GJ. Dose response studies using desferrioxamine and orally active chelators in a mouse model. *Scand J Haematol* 1986; 37 : 63-70.
5. Kontoghiorghes GJ, Nasseri-Sina P, Goddard JG, Barr JM, Nortney P, Sheppard LN. Safety of oral iron chelator L1. *Lancet* 1989; 334 : 457-458.
6. Huehns ER, Porter JB, Hider RC. Selection of hydroxypyridin-4-ones for the treatment of iron overload using in vitro and in vivo models. *Hemoglobin* 1988; 12 : 593-600.
7. Porter JB, Hoyes KP, Abeysinghe R, Huehns ER, Hider RC. Animal toxicology of iron chelator L1. *Lancet* 1989; 334 : 156.
8. Hoffbrand AV, Bartlett AN, Veys PA, O'Connor NTJ, Kontoghiorghes GJ. Agranulocytosis and thrombocytopinia in patient with Blackfan-Diamond anemia during oral chelator trial. *Lancet* 1989; 334 : 457.
9. Veys PA, Wilkes S, Al-Refaie FN, et al. The mechanism of agranulocytosis mediated by the oral iron chelator L1. *Br J Haematol* 1993; 84 (Suppl 1) : 64.
10. Mehta J, Singhal S, Revankar R, Walvalkar A, Chablani A, Mehta BC. Fatal systemic lupus erythematosus in patient taking oral iron chelator L1. *Lancet* 1991; 337 : 298.
11. Bartlett AN, Hoffbrand AV, Kontoghiorghes GJ. Long-term trials with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (I1). II. Clinical observations. *Br J Haematol* 1990; 76 : 301-304.
12. Olivieri NF, Koren G, Hermann C, et al. Comparison of oral iron chelator L1 and desferrioxamine in iron-loaded patients. *Lancet* 1990; 336 : 1275-1279.
13. Olivieri NF, Koren G, Freedman MH, Roifman C. Rarity of systemic lupus erythematous after oral iron chelator LI. *Lancet* 1991; 337-924.
14. Al-Refaie, Wonke B, Hoffbrand AV, Wickens DG, Nortey P, Kontoghiorghes GJ. Efficacy possible adverse effects of the oral chelator 1,2 dimethy 1-3 hydroxypyrid-4-one (L1) in thalasemia major. Blood 1992; 80: 593-599
15. Agarwal, Viswanathan C, Ramanathan J, et al. Oral iron chelation with LI. *Lancet* 1990; 335-601.
16. Agarwal MB, Gupte SS, Viswanathan C, et al. Phase II trial 1-2, dymethy-3-hydroxyrid-4-one(LI the oral iron chelor in 52 patients of transfusion dependent thalassaemic. *Blood* 1990; 76 (Suppl 1): 52a.
17. Agarwal MB, Gupte SS, Viswanathan C, et al. Effective oral iron chelation in patient of thalassemi. Proceeding of the International Symposium cum Workshop on "Anemia in children," Bombay, February 1991, 13.1-13.3.
18. Agarwal MB, Gupte SS, Vasandani D, *et al.* Efficacy and safety of 1-2, dimethyl-3-hydroxpyrid-4-on (L_1)as an oral iron chelator in patients of beta thalassaemia major with iron overload. *J Assoc Physic India* 1991; 39 : 669-72.
19. Agarwal MB Gupte SS, Viswanathan C, *et al.* Long-term assessment of efficacy and safety of L_1, an oral iron chelator, in transfusion dependent thalassaemia: Indian trial. *Br J Haematol* 1992; 82 : 460-6.
20. Berdoukas V. Antinuclear antibodies in patients taking L_1.*Lancet* 1991; 337-672.
21. Hahn BH. Systemic lupus erythematosus. In Wilson JD, Braunwald E, Isselbacher KJ, *et al* : "Harrison's principles of Internal Medicine," 12th ed. New York : McGraw-Hill, 1990; pp 1432-1437.
22. Mehta J, Chablani A, Reporter R, Singhal S, Mehta BC. Occurrence of autoantibodies in thalassemia major and their possible relationship with oral iron chelator L_1. *Br J Haematol* 1993; 84 (Suppl 1) : 64.
23. Mehta J, Singhal S, Mehta BC. Oral iron chelator L_1 and autoimmunity. *Blood* 1993; (in press).
24. Berdoukas V, Bentley P, Frost H, Schnebli HP. Toxicity of oral chelator L_1. *Lancet* 1993; 321 : 1088.
25. Hershko C. Development of oral iron chelator L_1. *Lancet* 1993; 341 : 1088-9.