PATIENTS WITH transfusional iron overload urgently need a safe, inexpensive, orally active chelating agent that effectively promotes iron excretion. Because humans lack a physiologic means of eliminating excess iron, iron contained in transfused red blood cells (RBCs) progressively accumulates and eventually damages the liver, heart, pancreas, and other organs; death is usually from cardiac failure.1 The physiologic limitation that prevents the elimination of accumulated iron can be circumvented by treatment with a chelating agent capable of complexing with iron and permitting its excretion. The only iron-chelating agent now available for clinical use is a bacterial siderophore first introduced 3 decades ago,2 deferoxamine B, a trihydroxamic acid produced by Streptomyces pilosus. Clinical trials with deferoxamine (Desferal; Ciba-Geigy Ltd, Basel, Switzerland) have now documented the effectiveness of iron chelation as a therapeutic approach to iron overload,3-5 demonstrating that regular chelator treatment can decrease the body iron burden,6 ameliorate organ dysfunction,7-11 and improve survival.12,13 Although a variety of toxic side effects have now been recognized,14-17 especially with intensive therapy, deferoxamine has been a remarkably safe drug, even with near life-long use in some patients. Unfortunately, deferoxamine is so poorly absorbed after being given by mouth that parenteral administration is required and the drug is so rapidly eliminated that prolonged infusions are needed to produce a therapeutic effect. The resultant need for subcutaneous or intravenous (IV) administration by portable infusion pump for 9 to 12 hours each day limits compliance in many patients and, together with the high cost of the drug, makes the use of deferoxamine impractical or impossible in many parts of the world where chelators are most needed. Thus, despite proven efficacy under favorable conditions, the problems of toxicity in some patients, the high cost, and the difficulties of complying with the requirement for prolonged parenteral administration have mandated a search for a safe, inexpensive, and orally active chelating agent that can be administered by the oral route for the chronic treatment of iron overload. The member of this family of chelators that has received the most extensive evaluation in animal and human studies is 1,2-dimethyl-3-hydroxypyridin-4-one (hereafter, L1; also known as CP20 and DMHP). Physicochemically the drug is a white crystalline solid, readily soluble in water (16 to 18 mg/mL at 24°C). L1 is a neutral molecule that forms a neutral red 3:1 chelator-iron complex7 at pH 7.4. The shelf life of the agent has been estimated to be more than 3 years and the drug is stable in both acidic (pH < 1) and basic (pH > 12) solutions; the chelator-iron complex is also stable in solution for prolonged periods. L1 can mobilize iron from ferritin, hemosiderin, lactoferrin, and diferic transferrin18,19, and inhibits free radical formation catalyzed by iron. L1 inhibits cyclooxygenase, lipoxygenase, ribonucleotide reductase, and their enzymatic pathways.20 This agent has been found to inhibit DNA synthesis in lymphocytes and in neuroblastoma cell lines in vitro.21

The efficacy of L1 in removing iron in animals has been examined in rodents and, recently, in the Cebus monkey. In the mouse, a single dose of 200 mg/kg of intraperitoneal deferoxamine, intraperitoneal L1, and oral L1 each produce a similar increase in iron excretion.22 A variety of further studies in mice, rats, and rabbits yielded variable results.23-30 More recently, investigations in the hypertransfused rat found that L1 and deferoxamine were comparable in chelating efficiency. Using selective radioiron probes, urinary iron excretion of iron mobilized by L1 was found to be derived from reticuloendothelial cells while a portion of the iron mobilized from reticuloendothelial cells and all of the iron derived from hepatocytes was excreted through the bile.31 Because studies in primates may predict results in humans more reliably than studies in rodents, the effect of

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L1 was examined in the *Cebus* monkey in which iron overload (500 mg Fe/kg) had been produced by IV iron dextran. A comparison was made between doses of equivalent iron-binding capacity of deferoxamine administered subcutaneously (150 μmol/kg or 100 mg/kg) and of L1 administered orally (450 μmol/kg or 63 mg/kg). Chelation efficiency (observed total iron excretion/theoretical total iron excretion with chelator, expressed as a percent) with L1 (2.1%) was less than half that observed with deferoxamine (5.5%). In contrast to the rodent studies, where both urinary and biliary iron excretion were observed, iron excretion produced by L1 in the monkey was exclusively urinary. At the dose examined, iron balance studies found that deferoxamine, but not L1, could maintain a negative iron balance.32

Toxicologic evaluation of L1 in animals has not followed the customary pattern. Before administration of an investigational new drug to humans in the United States, the Food and Drug Administration generally requires a series of acute, subacute, and chronic studies in at least two species (one rodent, one nonrodent) meeting Good Laboratory Practice standards to precede, in turn, acute, subacute, and chronic studies in humans. Requirements are not inflexible and exemptions may be granted, but the intent is that studies in animals precede those of comparable duration in humans so as to warn investigators of potential toxic side effects of a new drug. Regulatory authorities in most countries have similar requirements as well as provisions for exceptions and exemptions. L1 did not receive full formal toxicologic evaluation before being given to human subjects; permission to administer L1 to patients in other countries was initially granted on the basis of limited toxicity studies in rodents. The available published data may be briefly summarized. In acute toxicity studies in mice, the LD₅₀ after intraperitoneal injection has been estimated to be about 1,000 mg/kg;32 in rats the median lethal dose with intraperitoneal injection is estimated to be about 650 mg/kg. More limited data on the subacute toxicity of L1 are also from other published studies in rodents. At a dose of 200 mg/kg daily by intraperitoneal injection, anemia, leukopenia, and thrombocytopenia have been reported in mice;32 anemia and leukopenia, but not thrombocytopenia, were reported in rats. Other side effects of L1 administration that have been noted but not reported in full are hypersalivation, prolonged anesthesia with barbituates, and electroretinographic changes.32,33,36,37

To date, the only completed toxicologic evaluation of L1 in animals that was performed under Good Laboratory Practice standards was sponsored by the National Institute of Diabetes, Digestive and Kidney Diseases of the National Institutes of Health. In a formal toxicity study, pairs of male and female dogs were administered doses of 0, 25, 50, 100, 200, 400, and 600 mg/kg each, once daily by gelatin capsule for 14 days. In both males and females, deaths occurred only at the highest dose of 600 mg/kg/d. At doses of 400 mg/kg/d or more, gastrointestinal lesions and adrenal hypertrophy developed in both males and females; gonadal atrophy was observed only in males. At doses of 200 mg/kg/d or more, growth retardation, bone marrow atrophy, reticulocytopenia, leukopenia, and thrombocytopenia developed. Further formal toxicologic evaluations by Ciba-Geigy under Good Laboratory Practice conditions are in progress but some preliminary information has been made available (personal communication, Drs R. Andreatta and P. Bentley, Ciba-Geigy, Ltd, Basel, Switzerland). In 3-month toxicity studies in rats and *Cynomolgus* monkeys, a dose-dependent effect of L1 was noted on structural blood elements, especially the white blood cells, that was considered suggestive of bone marrow depression. In the group of monkeys receiving the highest dose (initially 250 mg/kg/d but subsequently reduced to 150 mg/kg/d by day 20), 5 of 13 animals died. Furthermore, in an exploratory reproduction toxicology study in rats, marked embryotoxicity was noted at doses of 100 mg/kg/d and higher; the pattern of embryopathy resembled that of a cytotoxic drug.

Studies of L1 in humans began in 1987 in London and have now been extended by other investigators in Toronto, Bern, Bombay, and elsewhere. No clinical studies of L1 have been performed in the United States. Later reports from London,44,45 including the results described in this issue of *Blood*,19 and from Toronto46 have provided information on patients who have taken L1 for 1 year or more. Limited data are available on the pharmacokinetics of L1 in humans. Absorption is apparently from the stomach (half-life 1 to 5 minutes) with the drug appearing in the blood within 5 to 10 minutes. Importantly, glucuronation is the major route of L1 metabolism and almost all the drug administered is recovered in the urine as the glucuronide (which is not an iron chelator) or as the iron-L1 chelate.47 The half-life of serum elimination has been reported as 47 to 134 minutes. Within 5 to 6 hours, 85% to 90% of the drug was eliminated from the serum. Maximum serum concentrations were 132 ± 44 μmol/L with a 3-g dose (35 to 75 mg/kg) in one study. In another study with a dose of 25 mg/kg, the peak serum concentration occurred 45 to 90 minutes after ingestion of L1 and ranged from 51 to 150 μmol/L. Elimination half-life varied between 117 and 237 minutes.44,45

The published data available on the safety of L1 include the studies listed above, case reports from investigators,49-50 and communications from the International Collaborative Study Group on Oral Iron Chelators.51 In reviewing the reported adverse reactions, it should be recognized that no standard international source of L1 has been available; each of the groups of investigators in London, Toronto, Bern, and Bombay has arranged for the preparation of their own supply of the chelator. Mild adverse effects associated with L1 administration described by Al-Refaie et al19 include dermatologic changes associated with zinc depletion, minor gastrointestinal complaints, transient liver function abnormalities, and musculoskeletal symptoms, as have been reported earlier by these45 and other investigators. Three patients developed low titers of autoantibodies during the trial but without apparent relation to musculoskeletal symptoms; the significance of earlier reports of autoantibodies is unclear.43,49,50,51 In none of the clinical studies reported to date have abnormalities in neurologic, retinal, auditory, cardiac, or renal function been detected nor have adrenal hypertrophy, gonadal atrophy, or lipid abnormalities been recognized.

The most serious adverse reactions that have been reported to occur with L1 have been the two cases of...
agranulocytosis that have been reported from London. The first occurred in 1 of 13 participants in a trial of L1, a 28-year-old woman with Blackfan-Diamond anemia who had received L1 at a dose of 105 mg/kg/d for 6 weeks.\textsuperscript{55,66} Agranulocytosis, with septicemia and shock, and marked thrombocytopenia developed in association with a severely hypoplastic bone marrow. Platelets returned to their usual levels in 10 days, neutrophils reappeared after 17 days and returned to their usual levels in 36 days. The second case occurred in 1 of 11 participants in the trial of L1 described in this issue,\textsuperscript{19} a 20-year-old woman with thalassemia major who had also received L1 at a dose of 105 mg/kg/d for 6 weeks. Agranulocytosis, in association with a cellular bone marrow with absent white blood cell precursors, persisted for 7 weeks. Agranulocytosis with L1 has not developed in hypoplastic bone marrow. Platelets returned to their usual levels in 36 days.

Many reports of increases in urinary iron excretion after therapy.

In this issue,\textsuperscript{19} a 20-year-old woman with thalassemia major had received L1 at a dose of 105 mg/kg/d for 6 weeks. Agranulocytosis that have been reported from London. The second case occurred in 1 of 11 participants in the trial of L1 described in this issue,\textsuperscript{19} a 20-year-old woman with thalassemia major who had also received L1 at a dose of 105 mg/kg/d for 6 weeks. Agranulocytosis with L1 has not developed in hypoplastic bone marrow. Platelets returned to their usual levels in 36 days.

The efficacy of L1 in reducing the body iron burden in patients with iron overload has been uncertain, despite the many reports of increases in urinary iron excretion after administration of the chelator.\textsuperscript{19,39-44} In a comparative study of urinary iron excretion in five patients, a dose of 75 mg L1/kg (180 μmol iron-binding equivalents/kg) resulted in a urinary iron excretion of 26.7 mg Fe/d that was similar to the 24.9 mg Fe/d produced by a dose of 50 mg deferoxamine/kg subcutaneously (76 μmol iron-binding equivalents/kg).\textsuperscript{41} Restated, this study found that, in terms of iron-binding equivalents, more than twice the dose of L1 was required to produce the same urinary iron excretion as deferoxamine. This comparison of urinary iron excretion did not take into account any fecal iron excretion produced by deferoxamine, which may constitute a substantial fraction of the total deferoxamine-induced iron excretion. If total (fecal + urinary) iron excretion with deferoxamine is considered, then the effect on iron excretion of a dose of 75 mg L1/kg may correspond to a dose of subcutaneous deferoxamine of about 30 to 40 mg/kg.

Two earlier studies found no consistent decrease in the serum ferritin concentrations after treatment with L1. In London, in 13 patients treated with a variety of doses of L1 for from 1 to 15 months, serum ferritins fluctuated but were unchanged overall.\textsuperscript{44} Similarly, in Bern, eight patients treated with L1 in doses of 55 to 80 mg/kg/d from 4 to 10 months as a whole had no decrease in serum ferritin concentrations. The present study by Al-Refaie et al\textsuperscript{19} using a higher dose of about 100 mg/kg/d, now reports a statistically significant decrease in serum ferritin concentrations in 10 patients. However, their Figure 3 makes clear that only those patients with concentrations greater than 5,000 μg/L, presumably those with the greatest body iron burdens, had a substantial decrease in serum ferritins. Using changes in serum ferritin to assess the effectiveness of a chelating agent is problematic because of factors that can alter the serum ferritin independently of changes in body iron stores, such as ascorbate deficiency, inflammation, and liver disease.\textsuperscript{52} Direct evidence that L1 can decrease body iron has now been obtained by Olivieri et al\textsuperscript{53} in Toronto. In a 29-year-old man with thalassemia intermedia, L1, 75 mg/kg/d administered for 9 months resulted in a reduction in hepatic iron from 14.6 mg Fe/g liver, dry weight, to 1.9 mg Fe/g liver; the serum ferritin decreased from 2,174 μg/L to 251 μg/L.\textsuperscript{53} Furthermore, in a year-long study of 11 patients with thalassemia major, also treated with L1, 75 mg/kg/d, chemical measurements of iron in specimens of liver obtained by liver biopsy initially and at 12 months found a general pattern of decreased concentrations in patients with the highest initial hepatic irons, little change in those with intermediate levels, and some increases in those with the lowest initial values.\textsuperscript{56}

Overall, the available evidence from studies in animals and from clinical trials suggests a delicate balance between safety and efficacy for L1. The subacute toxicity studies in rodents and dogs\textsuperscript{39} suggest that dose-dependent neutropenia is an important toxic effect of L1, and, together with the preliminary results from the toxicologic evaluation in Cynomolgus monkeys, indicate that risks of severe complications are present at doses of 150 to 200 mg/kg/d or less. The best assessment of efficacy in animals is derived from the studies in Cebus monkeys,\textsuperscript{32} suggesting that, for doses of equivalent iron-binding capacity, the chelation efficiency of L4 will be less than half that of subcutaneous deferoxamine. The limited experience with L4 in clinical trials thus far seems consistent with these expectations from studies in animals. The major adverse effect that has been observed is severe agranulocytosis, which has developed in 2 of 24 patients\textsuperscript{19,45} treated with doses of 100 mg/kg/d, or more, but in 0 of 19 patients who have received doses of about 75 mg/kg/d or less.\textsuperscript{46} Judging (1) from measurements of urinary iron excretion\textsuperscript{41} and (2) from a year-long comparison of patients treated with L1 and age-matched patients treated with subcutaneous deferoxamine,\textsuperscript{46} for doses of equivalent iron-binding capacity, the efficacy of L1 in producing iron excretion seems to be somewhat less than half that of deferoxamine. If toxicity was eventually found to place an upper limit of about 75 mg/kg/d as the maximum tolerable dose of L1 for the treatment of iron overload, the available data suggest that the effectiveness of this dose might roughly correspond to that of deferoxamine at a dose of about 30 to 40 mg/kg/d.

In this issue of Blood, Al-Refaie et al\textsuperscript{19} call for further clinical trials of L1 to determine the incidence and severity of adverse effects. Trials in London, Toronto, and Bern are continuing, other clinical studies are apparently in progress in several countries,\textsuperscript{33} and plans for more extensive trials in Europe have been announced (see below). No clinical studies of L1 have been performed in the United States, although the US Food and Drug Administration (FDA) has indicated that such trials could be approved. In August 1991, the data summarized above were reviewed by the US FDA (Division of Gastrointestinal and Coagulation Drug Products, Office of Drug Evaluation I, Center for Drug Evaluation and Research) at a “pre-IND” (pre-Investigational New Drug) meeting. The purpose of the meeting was to determine if sufficient preclinical and clinical data were available to support a Phase II prospective, randomized
clinical trial in patients with thalassemia major that would compare L1 given orally with deferoxamine administered subcutaneously. It was recognized that the current preclinical data for L1 did not meet the usual FDA guidelines for a Phase II study and that agranulocytosis and other adverse reactions with the drug had been reported. Nonetheless, the representatives of the FDA judged that the observed toxicities would not preclude a Phase II trial while emphasizing that closely monitored, clearly defined safety parameters would be required for such a study. The FDA representatives indicated that, in principle, a Phase II trial could be allowed to proceed and could serve as the basis for drug approval, provided that the protocol included (1) clearly defined endpoints for the clinical trial, (2) pre-established safety guidelines for stopping the study if indicated, and (3) careful monitoring of safety parameters with a mechanism for prompt reporting of problems. Because the formulation of the drug to be tested is related to safety and efficacy, an essential requirement for a trial would be the availability of a carefully defined product from an established source with full chemistry, manufacturing, and control data. Finally, the representatives of the FDA recognized the potential benefits of an orally active iron chelator and noted that L1 would qualify for “orphan” drug status.

In sum, the data presented by Al-Refaie et al.\textsuperscript{9} in this issue of Blood demonstrate that oral administration of L1 to patients with iron overload can produce clinically useful amounts of iron excretion and, in conjunction with the studies of Olivieri et al.\textsuperscript{46,53} decrease body iron stores. Whatever the ultimate clinical usefulness of this particular chelator, the experience with L1 has unequivocally established the feasibility of effective iron chelation therapy with orally active agents. This accomplishment should serve to stimulate the search for still safer and more effective iron chelators that will remain active when administered by mouth. The extent to which drug toxicity, especially agranulocytosis, will limit the clinical usefulness of L1 remains to be determined in clinical trials.

The Third NIH-Sponsored Symposium on the Development of Iron Chelators for Clinical Use has recently provided an up-to-date view of the development and potential usefulness of iron-chelating agents. In the dozen years since the last conference, remarkable progress has been made both in understanding the basic biochemical pharmacology of iron chelation and toxicity and in the ability of pharmaceutical chemists to design, synthesize, and evaluate iron-chelating agents. While the use of iron chelators for the therapy of iron overload remained a central theme of the Symposium, other potential applications of iron-chelating agents have emerged, including the prevention of cellular damage resulting from inflammatory reactions involving toxic oxygen products, protection from ischemia-reperfusion injury, and the chemotherapy of malaria.

As at the last Symposium, deferoxamine remains the only clinically available iron-chelating agent. While recognizing the successes of this agent in the management of patients with iron overload, it was also recognized that deferoxamine has proven inadequate for many needful patients because of neurotoxicity or other toxicity, the problems associated with prolonged parenteral administration, or combinations of these and other factors, such as cost. Deferoxamine therapy is failing in many patients with thalassemia major (Cooley’s anemia) or other inherited forms of anemia, in others with sickle cell anemia who require transfusion for the prevention of stroke or other complications, and in still other patients with acquired aplastic or refractory anemias. In these patients, repeated RBC transfusions will inevitably lead to toxic and eventually lethal accumulations of iron and an alternative to deferoxamine is urgently needed for them.

The Symposium provided a catalog of the resources now assembled for the development of new iron chelators and of the current status of candidate agents. Representatives of Ciba-Geigy, Ltd (Basel, Switzerland), the manufacturer of deferoxamine (Desferal), described a research program with three approaches: (1) production of novel formulations and derivatives of deferoxamine to improve oral absorption; (2) synthesis of deferoxamine prodrugs to either improve absorption or serve as injectable “depot” forms; and (3) development of new iron-chelating agents, including desferrithiocin analogs and L1. With respect to the latter chelator, Ciba-Geigy made the decision in late 1991 to develop L1 under an option agreement with the British Technology Group. Development is proceeding to provide the necessary preclinical data needed by health authorities for approval of clinical studies, to supply full chemistry, manufacturing, and quality control data, and to produce sufficient quantities of the drug for investigative use. This effort is scheduled to be completed by the fourth quarter of 1992 and adequate supplies of L1 should then be ready for clinical trials.

Investigators described other plans for the development of new iron-chelating agents. At least three other orally active iron-chelating agents are being examined clinically: (1) another hydroxyxypiridone, 1,2-diethyl-3-hydroxyxypyrindin-4-one (CP94); (2) N,N-bis (2-hydroxybenzoyl) ethylenediamine (HBED); and (3) a prodrg form of pyridoxal isonicotinoyl hydrazone (PIH). In addition to new desferrithiocin analogs, a variety of new hydroxyxypyrndones have been synthesized and are now being evaluated. Finally, in the United States, federal government agencies are preparing to facilitate the development of oral iron-chelating agents. Because iron chelators qualify for “orphan” drug status, the Office of Orphan Product Development at the FDA will provide assistance in regulatory matters, help identify pharmaceutical sponsors for promising agents, and has limited funds to support clinical studies. At the NIH, the National Institute of Diabetes and Digestive and Kidney Diseases is currently awarding a contract to continue its program of toxicologic evaluation of candidate chelators in animals. Two years ago, the Advisory Council of the National Heart, Lung and Blood Institute approved plans to provide support for long-term clinical studies of the safety and efficacy of orally active iron-chelating agents when suitable candidate agents become available. Both Institutes can offer support for relevant investigator-initiated (R01) proposals for the development of iron chelators and for related studies of iron metabolism.
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