IN VIVO TESTING OF ORAL IRON CHELATORS INTENDED FOR CLINICAL USE

To the Editor:

The effectiveness of 1,2-dimethyl-3-hydroxy pyridine-4-one (L1) in increasing iron excretion in iron-loaded patients improved the prospects for replacing deferoxamine (DF) with an oral chelating drug.1,2 L1 belongs to a novel group of iron chelators, the \( \alpha \)-ketohydroxypyridines (KHP),3 which are cheap to prepare and orally active. Recently, a detailed article by Porter et al.4 on in vivo studies in mice with KHP iron chelators concluded that several of these compounds appear to be more effective and less toxic than L1 and DF. However, the results of that study contradict earlier findings in the same and other models,4,5 while insufficient references and the use of a single, experimental animal model question the validity of their conclusions. For example, the synthesis and purification of almost all the KHP described4 are not found in references 11 and 12 but can be found in other publications.6,7 Reference 18 was not submitted or published, nor does reference 17 describe the distribution of \( ^{59} \text{Fe} \) from \( ^{59} \text{Fe} \) transferrin.8 However, the results of that study contradict earlier references 11 and 12 but can be found in other publications.7

The drawbacks of the mouse iron excretion model used4,5,7,8 are the inability to measure the total iron that is \( ^{59} \text{Fe} \) and carrier iron (from iron dextran), both of which are distributed in different compartments and which may offer variable accessibility to different chelators in animals.9,10 In mice, the distribution of \( ^{59} \text{Fe} \) 2 weeks after the intravenous (IV) administration of \( ^{59} \text{Fe} \) lactoferrin is mainly in hemoglobin9 and not in the liver, as suggested.7 This finding may be relevant to the observations that doses of 50 mg/kg are not effective in this model1 but are effective in humans, and also that the site of iron excretion varies with the animal species and iron-loading procedures used.12

The assumption that highly hydrophilic compounds may be orally inactive1 is invalid because pharmacokinetic studies have shown almost 100% recovery of oral L1 in the urine of humans.13 The oral efficacy of L1 in increasing iron excretion in the mouse model is equivalent or higher to the other more lipophilic homologous chelators with the exception of 1-allyl-2-methyl-3-hydroxypyrid-4-one (L1NAll),14 which is the most effective of the series but, like the other lipophilic chelators, is more toxic than L1.

Another major discrepancy in the paper of Porter et al.4 is the acute toxicity study where the methodology of repeated administrations every 48 hours in unspecified number of mice and doses as well as the unspecified number of deaths is incorrectly related to LD50. In that study, mice may have died with one or more single doses if they were left for over 48 hours. The incorrect “LD50” reported1 should, therefore, be regarded as an overestimation. This may explain the lower intraperitoneal lethal dose (LD50) observed by all these chelators in rats4 where L1 was the least toxic of the series. It can also explain the lack of correlation between lipophilicity and acute toxicity in their mice study,5 which was clearly demonstrated elsewhere.10 Based on this discrepancy it is likely that the estimation of the therapeutic safety margin of all the KHP they have tested was incorrect.

The higher toxicity margin of lipophilic chelators such as the 1,2-diethyl-3-hydroxy pyridine-4-one (EL1NNeOEt or C94) and 1-(2-methoxyethyl)-3-hydroxy pyridine-4-one (L1NMeOEt or C52) by comparison with hydrophilic chelators such as L1 and DF has also been shown in the long-term oral administration of 200 mg/kg doses, 5 days a week in rats.10 All the rats treated with lipophilic derivatives died within 3 (EL1NNeOEt) and 5 (L1NMeOEt) months, but none with L1 and less than 20% with DF. The high toxicity margin of EL1NNeOEt has now been confirmed by the same authors5 who suggested that oral administration of doses only lower than 50 mg/kg for a maximum 28 days may have acceptable level of toxicity in rats. However, in our more extended studies (unpublished), oral EL1NNeOEt at 50 mg/kg, 5 days a week causes 50% mortality in rats within 3 months. In addition, the leucopenia observed by oral L1 at 200 mg/kg11 in long-term studies in rats is a side effect observed by most L1-substituted-2-alkyl-3-hydroxy pyridine-4-ones, including EL1NNeOEt. However, this latter chelator, unlike L1, causes convulsion in rats, indicating central nervous system involvement.

The chronic treatment of transfusional iron overload by an oral KHP chelator will require the daily administration of doses higher than 50 mg/kg to bring patients to negative iron balance. The success of DF with regards to low toxicity at high doses in transfusional iron-loaded patients appears to be related to its and its iron complex hydrophilicity, which is also apparent with L1 in animals and humans. The use of lipophilic chelators in humans may be desirable in short-term studies, but these will have to be administered at much lower doses and their safety during long-term administration is questionable.

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**RESPONSE**

We note Kontogiorghes' letter but regard many of the points made to be invalid and unsubstantiated and stand by the detailed results and overall conclusions of our report.1 The thrust of our work has been to try to establish the relationship between structure, lipid solubility, efficacy, and toxicity of the 3-hydroxypyridin-4-ones as a group to help in the design of new compounds and selection of the most promising available for further development. 12

Our finding that the dimethyl-compound (CP20 or L1) (the structures of the CP series of compounds are given in reference 1) is less effective in mobilizing iron when compared with the diethyl derivative (CP94) has recently been confirmed by others using rats.3 We agree that measurement of total iron removal rather than just 59Fe iron should be performed, and further suggest that longer-term iron removal studies are also required. With these points in mind, we gave four 3-hydroxypyridin-4-ones, including CP20 (L1) and CP94, as well as desferrioxamine, as a single daily dose of 200 mg/kg IP to iron-overloaded and non-iron-loaded mice for 60 days. The preliminary results of this study were presented at the 1989 meeting of the American Society of Hematology,4 as well as to the British Society for Haematology.5 These results again showed the same trends in terms of iron removal from the liver. We believe that any extra toxicity of CP94 over CP20 reported by Kontogiorghes6 may be related to the use of non-iron-loaded animals because iron is known to protect against chelator toxicity.12

It is counterproductive to review the references to Kontogiorghes' own group cited in his letter in detail because they are available for all to study. However, his references 8 and 9 contain no work on CP94, while his reference 10 was published after our paper. The relative hydrophobicities of a number of hydroxypyridin-4-ones are given in Table 1 of our paper.6 It can be seen that the free ligand, CP20 (L1) is not unduly hydrophilic; it passes across cell membranes and is effective when administered orally. The much more hydrophilic compound (CP40) permeates cell membranes more slowly and would therefore be expected to be only minimally effective orally. Another point Kontogiorghes makes is related to the distribution of 59Fe after injection of 59Fe-lactoferrin into iron-overloaded mice, asserting that it is mainly in hemoglobin. There are no such data in his reference 8, which quotes the work of Van Snick et al.7 This latter report gives some relevant data in non-iron-loaded animals, at day 11 the amount in hemoglobin being 11%.

The use of a 'modified' LD50 reduces the number of animals used in such experiments in keeping with modern guidelines issued by the home office in the UK. The number of animals used in each of our modified LD50 experiments is specified in Table 1 of our paper.8

We agree that reference 17 on page 2390, column 2, line 3 of our report should have read 18. The methods for the syntheses of the 3-hydroxypyridin-4-ones are well established,9 the use of the benzylcarbonyl function being introduced by Hare et al in 1974.10 The methodology used by our group is provided in reference 11 of our report.11

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In vivo testing of oral iron chelators intended for clinical use [letter; comment]

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