IN VIVO TESTING OF ORAL IRON CHELATED INTENDED FOR CLINICAL USE

To the Editor:

The effectiveness of 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in increasing iron excretion in iron-loaded patients improved the prospects for replacing desferrioxamine (DF) with an oral chelating drug. L1 belongs to a new group of iron chelators, the alpha-ketohydroxypyridines (KHP), which are cheap to prepare and orally active. Recently, a detailed article by Porter et al. 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, 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 described are not found in references 11 and 12 but can be found in other publications. Reference 18 was not submitted or published, nor does reference 17 describe the distribution of [59Fe] from Fe lactoferrin. These compounds appear to be more effective and less toxic than L1 and DF. However, the results of that study contradict earlier references and can be found in other publications.

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

The assumption that highly hydrophilic compounds may be orally inactive is invalid because pharmacokinetic studies have shown almost 100% recovery of oral L1 in the urine of humans. 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), 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. 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’ reported should, therefore, be regarded as an overestimation. This may explain the lower intraperitoneal lethal dose (LD50) observed by all these chelators in rats where L1 was the least toxic of the series. It can also explain the lack of correlation between lipophilicity and acute toxicity in their mouse study, which was clearly demonstrated elsewhere. 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-hydroxypyrid-4-one (EL1NEt or C94) and 1-(2-methoxyethyl)-3-hydroxypyrid-4-one (LINMeOEt 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. All the rats treated with lipophilic derivatives died within 3 (EL1NEt) and 5 (LINMeOEt) months, but none with L1 and less than 20% with DF. The high toxicity margin of EL1NEt has now been confirmed by the same authors 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 EL1NEt at 50 mg/kg, 5 days a week causes 50% mortality in rats within 3 months. In addition, the leukopenia observed by oral L1 at 200 mg/kg in long-term studies in rats is a side effect observed by most 1-substituted-2-alkyl-3-hydroxypyrid-4-ones, including EL1NEt. 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.

GEORGE KONTOGHIORGES
Department of Haematology
School of Medicine
The Royal Free Hospital
University of London
London, UK

REFERENCES


From www.bloodjournal.org by guest on September 24, 2017. For personal use only.
We note Kontoghiorghes’ 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. 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.  

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.1 We agree that measurement of total iron removal rather than 59Fe after injection of 59Fe-lactoferrin into iron-overloaded mice, asserting that it is mainly in hemoglobin, is counterproductive to review the references to Kontoghiorghes’ 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 methodology used by our group is provided in reference 11 of our report.  

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.  

We agree that reference 17 on page 2390, column 2, line 3 of our report 1 should have read 18. The methods for the syntheses of the 3-hydroxypyridin-4-ones are well established, the use of the benzylcarbonyl function being introduced by Hare et al in 1974. The methodology used by our group is provided in reference 11 of our paper.  

J.B. PORTER  
E.R. HUEHNS  
Department of Clinical Haematology  
University College and Middlesex School of Medicine  
R.C. HIDER  
Department of Pharmacy  
King’s College  
London, UK

REFERENCES

12. Kontoghiorghes GJ: Assessment of oral 1,2-dimethyl-3-hydroxyprid-4-one (L1) in different ferrikinetic models in animals and humans. Br J Haematol 77:440, 1990  

RESPONSE


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

G Kontoghiorghes