



Evaluation of Liver Toxicity of 2-Methyl-3-Hydroxypyridin-4-one in Iron Overloaded Rats

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Abstract

Hydroxypyridinone iron chelators are currently the main candidates for development of orally active iron chelating alternatives to desferrioxamine (DFO). In the present study, the relative efficacy and liver toxicity of a bidentate chelator, 2-methyl-3-hydroxypyridin-4-one (MHPO), was studied in iron overloaded rats and compared with those of DFO. For iron overloading, rats received i.p. injections of 100 mg/kg of iron-dextran twice a week for 4 consecutive weeks. They were allowed for equilibration of iron after overloading for 15 days. Then the rats received i.p. injections of 200 mg/kg/day of either MHPO or DFO for 15 days. At the end of this period, blood samples were taken and the iron and ferritin concentrations, and the total iron binding capacity (TIBC) were determined. The activities of SGOT, SGPT and ALP were analyzed by standard colorimetric kits. Serum values for iron, TIBC and ferritin were shown to have no significant differences after the administration of either MHPO or DFO in treated rats. SGOT and SGPT values were significantly reduced after the administration of MHPO. DFO, however, was only able to reduce SGPT with the same dose. There were no significant differences between two chelators with regards to ALP. After the administration of MHPO, skin rashes were observed in a way that rats could not move. In conclusion, this study confirms that MHPO is at least as effective as DFO at mobilizing iron, and reduces liver toxicity, however, with regard to other side effects such as its skin toxicity, further studies are required.

Keywords: Desferrioxamine; Hepatotoxicity; Hydroxypyridinones; Iron overload.

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1. Introduction

Patients who suffer from hemoglobinopathic disorders, such as β -thalassaemia major, are dependent on frequent blood transfusion

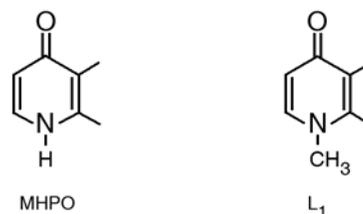
for survival. The result of continual blood transfusions is toxic accumulation of iron in the body. Chelation therapy is widely used to reduce toxic effects of accumulated iron. For the past 30 years, desferrioxamine (DFO) has been the only clinically useful drug available for this purpose [1, 2]. DFO, however, has disadvantages of being orally inactive and only causing enough iron excretion to keep

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pace with the transfusion regimens, when given either subcutaneously or intravenously over a period of 8-12 h several times a week. Moreover, DFO has serious side effects, including local skin reactions, hearing loss, neurotoxicity, nephrotoxicity, pulmonary toxicity, growth retardation and local infections [3-7].

Hydroxypyridin-4-one (HPOs) iron chelators are currently the main candidates for development of orally active iron chelating alternatives to DFO [8-10]. The only iron-chelating agent from the HPOs that has been chosen for initial studies in humans is deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one also known as L_1 or DMHP). Deferiprone is a neutral molecule that forms a neutral 3:1 chelator-iron complex at pH 7.4 [11]. L_1 is licensed in India for the treatment of iron overload. Several studies have examined the efficacy of L_1 in patients with thalassemia major [12]. Doses of 75 mg/kg/day or more caused negative iron balance and levels of urinary iron excretion that was similar to those in patients given standard dose of DFO [13]. The long-term efficacy of L_1 therapy in patients reported decrease in mean serum ferritin concentrations [14, 15]. However, there is evidence to suggest that this iron-chelating agent has serious side effects (including agranulocytosis, neutropenia, arthropathy, gastrointestinal disorders, and zinc deficiency) not characteristic of the series of compounds as a whole [16, 17]. However, the choice of which compound should be developed for use in humans is not yet clear.

2-Methyl-3-hydroxypyridin-4-one (MHPO), is a derivative of HPOs which its ability to cross cell membranes has been determined by measuring partition coefficient (k_{part}) between an organic phase (n-octanol) and water buffered to pH 7.4. It has been established that a k_{part} for a chelator in the range of 0.2-1.0 may facilitate its ability to penetrate cell membranes and yet show no acute toxicity [18-20]. The k_{part} values of MHPO and its related iron complex are 0.320 and 0.004, respectively [20]. Both L_1 and MHPO and their iron complexes are neutral.



Because there is evidence both with DFO [21, 22] and the hydroxypyridinones [23] that iron chelators protect against some of the toxic effects of iron overload, in the present study, we compared toxicity of 2-methyl-3-hydroxypyridin-4-one in normal and iron overloaded rats.

2. Materials and methods

2.1. Chemicals

2-Methyl-3-hydroxypyridin-4-one was synthesized as previously described [11]. Briefly, the 3-hydroxy function of maltol (Pfizer, Sandwich, UK) was benzylated using benzyl chloride in an appropriate solvent/base system. The resulting pyranone was then converted to the corresponding pyridinone by reaction with aqueous ammonia, the product was isolated as the HCl salt. The benzyl group was removed by hydrogenation method to yield the final compound. The purity of compound was tested by elemental analysis. All other chemicals were obtained from Aldrich (Gillingham, UK) and were of analytical grade unless otherwise stated. The k_{part} values were determined using an automated continuous flow technique [20].

2.2. Animal maintenance and sample collection

Thirty-three male Sprague-Dawley rats weighing between 200-220 g divided into iron overloaded (n=30) and control (n=3) groups. Rats were placed in polypropylene cages with stainless steel lids at an ambient temperature of 25 ± 2 °C with a 12 h light/dark cycle. The animals had free access to standard pellet chow and drinking water. Body weights were measured weekly throughout the study period. For iron overloading, rats received

i.p. injections of 100 mg/kg of iron dextran (100 µl) twice a week for 4 consecutive weeks [24] while the control group received i.p. injections of normal saline. Fifteen days were allowed for equilibration of iron after overloading. To make sure that the rats are iron overloaded, three rats from each test and control groups were sacrificed and serum iron were measured by spectrophotometric method. After the equilibration period, iron overloaded rats were divided into three groups (9 rats in each group) and received either 0.2 ml of MHPO or DFO (200 mg/kg, i.p.) or normal saline daily for fifteen days. After this period, the rats were sacrificed by cardiac puncture under light ether anesthesia. The collected blood was centrifuged and the serum was used for enzymatic assay. Glutamate oxalacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) were analyzed by enzymatic method using standard kits (cat no: 10-503 Zist Chemist). Alkaline phosphatase (ALP) was assayed by a colorimetric method [25].

Concentrations of iron and total iron binding capacity (TIBC) were determined by a spectrophotometric method [26], and the concentration of ferritin was analyzed by immunoenzymometric assay (cat. Kp 331w Radim Kit) [27].

2.3. Statistical analysis

One-way analysis of variance (ANOVA), followed by the Tukey's test was used. A probability value of $p < 0.05$ was accepted as being statistically significant.

3. Results

Although weigh gain was observed in all groups (8.5 ± 2.5 g), this was less apparent with MHPO group (6.5 ± 2.00 g). No death was occurred immediately after the administration of chelators.

Table 1 shows the serum values for iron, TIBC and ferritin of iron overloaded and non-overloaded rats. As it was expected in iron overloaded rats, iron concentrations were increased and TIBC decreased significantly after i.p. administration of iron dextran for 15 days. Ferritin, however, did not show any significant differences. After administration of any chelators, no significant differences were shown for the serum values of iron, TIBC and ferritin (Table 2).

MHPO significantly reduced the activity of SGOT and SGPT. There were no significant differences between the two chelators with regards to the activity of ALP.

After the administration of the MHPO for 15 days, the skin rashes were observed in iron overloaded rats in a way that they could not move. Two rats died at this time, which may attributed either to the toxicity of drug or to the weight loss.

4. Discussion

Investigation of toxicity-efficacy relationship of putative oral iron chelators is important before commencing formal toxicity testing and clinical trial. A number of compounds have shown a promising effect of oral iron chelation over the past two decades, only to be withdrawn later because of unacceptable toxicity [28-30]. It is also very important to distinguish which toxic effects

Table 1. Serum concentrations of iron, TIBC and ferritin, in iron overloaded and non-iron overloaded rats after 15 days of administration of iron dextran (Mean \pm SD, n=3).

Parameters Compound	Iron (µg/dl)	TIBC (µg/dl)	Ferritin (ng/ml)
Normal saline	137 \pm 7	357 \pm 27	39 \pm 1.6
Iron dextrane (100 mg/kg)	215 \pm 13*	277 \pm 6*	41 \pm 2

* Significant difference from corresponding value in normal saline treated rats ($p < 0.05$).

Table 2. Serum concentrations of iron, TIBC, ferritin, SGOT, SGPT and ALP after 15 days of administration of MHPO and DFO to iron overloaded rats (Mean±SD).

Parameters Compound	Iron (µg/dl)	TIBC (µg/dl)	Ferritin (ng/ml)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Normal saline (100 µl)	188±2	373±8	39±2.5	168±9	64±15	71±6
MHPO (200mg/kg)	156±9*	340.6±13	38±3.7	126±11*	31±2.5*	55±4*
DFO (200mg/kg)	159±11*	342±14	39±2.5	150±13	44±3.5*	54±4.1*

* Significantly different from corresponding value in normal saline treated rats ($p < 0.05$).

are protected by iron chelation and which are independent of it, so that chelators are not rejected as too toxic simply because they have chelated iron effectively, or because toxicity is found in non iron overloaded animals. For these reasons, we chose to study the relative efficacy and toxicity of a bidentate hydroxypyridin-4-one over 15 days in iron overloaded rats and compared them with those of DFO, the only drug proven to be clinically effective. All of the compounds were administered i.p., as it has been previously shown that oral or i.p. administration of the selected hydroxypyridin-4-one resulted in similar iron excretion [23].

The finding that weight gain in both control and iron overloaded rats was not significantly different may suggest that these chelators could protect against the toxic effects of iron. This finding has important implications for the design of more detailed toxicity testing in animals and for clinical studies in human.

Iron overloading of experimental animals with iron dextran has been described in mice [23], gerbils [31], Cebus monkeys [32] and rats [33]. Iron is initially taken up by the reticuloendothelial system, but then equilibrates with the parenchymal system. Nonetheless, the iron-overloading procedure proved to be very effective in our study as the serum iron was increased significantly after the administration of iron dextran (Table 1).

It can be inferred from our data that iron overload induced hepatocellular injury as evidenced by significant increases in SGPT activity in rats. Furthermore, an increase in SGPT activity may also be attributed to the

prooxidant potential of iron to serve as a radical-based initiator of hepatic lipid peroxidation [34-36].

Although serum enzyme levels are not a direct measure of hepatic injury, they show the status of the liver function. Lowering enzyme level is a definite indication of hepatoprotective action of a drug. Protection of hepatic damage caused by either DFO or MHPO administration was observed by recording SGOT and SGPT levels in treated, iron overload and normal rats because serum transaminase, and serum alkaline phosphatase have been reported to be sensitive indicators of liver injury [37]. Disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane [38]. This causes decreased levels of SGOT, SGPT and alkaline phosphatase in the hepatic cells and increased level in serum. The activity of SGOT and SGPT were reduced significantly after the administration of MHPO. DFO, however, was only able to reduce SGPT level with the same dose. These results strongly support the significant hepatoprotective activity of both drugs because SGPT is more specific than SGOT as an indicator of hepatic damage since SGPT is a cytoplasmic enzyme found in very high concentrations in the liver, and SGOT is present in the cytoplasm as well as in the mitochondria and is rapidly inactivated [39].

Our results also show that the ferritin concentration did not change significantly either after the administration of iron dextran

or any drug treatment. This would be attributed to the homeostatic role of ferritin as a storage site for cellular iron [40].

The finding that the administration of the MHPO causes skin rashes in iron overloaded rats might be an important side effect of MHPO, however, more studies are needed to find out the mechanistic insight of this side effect.

In conclusion, the hepatic iron overload initiated by iron dextran described in this paper confirmed biochemical events that occur concurrent with histopathologic events of iron induced liver injury. This injury might be eradicated or lowered by DFO or MHPO administration. Administration of either MHPO or DFO for 15 days revealed that the iron overloaded rats responded with a significant improvement in hepatic injury, as indicated by biochemical variables (SGPT, ALP and SGOT serum levels). This could be attributed to iron chelation by drug, which might decrease superoxide anion and hydroxyl radical formation. Furthermore, the results of this study have shown that two chelators (MHPO and DFO) have no liver toxicity with doses of 200 mg/kg.

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