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 effectives 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
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 L1 or DMHP). Deferiprone is a neutral molecule that forms
a neutral 3:1 chelator-iron complex at pH 7.4
[11]. L1 is licensed in India for the treatment
of iron overload. Several studies have
examined the efficacy of L1 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 L1 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 L1 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 benzylation 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
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 hydroxypridin-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 suggests 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.

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).

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

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

References


