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A Prodrug Approach for the Transportation of Phytic Acid Derivatives inside Cells

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S u m m a r y. A prodrug of phytic acid, as a cholesteryloxy carbonyl derivative was synthesized and its pharmacodynamic effect on oxygen delivery inside the Red Blood Cells is discussed. The compound showed a P50 right shift of oxyhemoglobin dissociation curve both in in vitro and in vivo preliminary experiments.

INTRODUCTION

Phytic acid (Inositol hexaphosphate, IHP, 1, Scheme 1) is the most abundant form of phosphate in plants (1). This organic polyphosphate has also the property to tightly bind to human hemoglobin (2) Comparing to 2,3-diphosphoglycerate 2 (DPG), Scheme 1, which is the allosteric effector for humans, IHP binds hemoglobin with 1000 fold higher affinity (3). By binding to hemoglobin, IHP triggers a decrease of the O_2/hemoglobin affinity and subsequently leads to release of oxygen. Therefore, IHP due also to its abundance in nature, represents a good pharmaceutical candidate in the case of diseases characterized by a limited oxygen flow to organs or tissues (generally called ischemia or ischemic insult). In physiological conditions, IHP bears at least 7 charges, making it very difficult to be transported across the cell membranes.

IHP delivery has already been achieved using a liposome delivery system (4,5), electrophoration techniques (6) and a chemical approach (7) (through a synthesis of a library of IHP derivatives ionically bound to lipophilic and non lipidic ammonium or polyammonium salts). We now attempt an approach, called the prodrug approach, based on the idea that a linker covalently bound to IHP could facilitate the transport of the polyphosphate inside the Red Blood Cells. The covalent bond between the transport molecule and IHP should be at the same time strong enough to remain intact through the transportation process and weak enough to be hydrolyzed inside the Red Blood Cells, Scheme 2. Acyl phosphates (8a,b) and cholesteryloxycarbonyl derivatives (9) have been shown to meet these two requirements as exemplified by several nucleoside triphosphate prodrug studies.

The IHP-cholesterol derivative 4, Scheme 3, represented our first choice because cholesterols were found to be exchanged in a variety of biological and artificial membranes. The formation of this adduct as well as the existence in the literature of stable phosphate anhydride derivatives from reactions of phosphates with acid formats (9) and acyl anhydrides (10a,b) supported theoretically this initial attempt.

METHODS

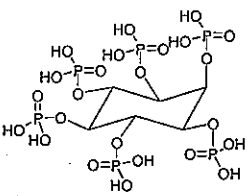
Synthesis and purification of cholesteryloxy carbonyl IHP derivative: A number of solvents and reaction conditions were investigated in order to obtain a clean and reproducible synthesis. The best conditions, that gave statistically one cholesterol moiety attached on IHP were found, when 1.5 eq of cholesteryl chloroformate was mixed with 1 eq of phytic acid (properly modified for solubility reasons), in a combination of THF and CH_3CN in a ratio 1:1 for 22h.

Biological evaluation of the IHP-derivative: The new IHP derivative was tested for pharmacody-

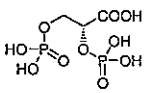
namic effects (shift of oxyhemoglobin dissociation curve) on murine free hemoglobin *in vitro*. Additionally, the compound was tested for its capacity of crossing the Plasma Membrane of Red Blood Cells and induce a rightwards shift of the P50 value of intracellular hemoglobin. Therefore, compound 4 was incubated with whole blood of different species, at different volume ratios and different osmolarities. The pH of the effector solutions was adjusted at 7.0-7.3. Finally, *in vivo* experiments were performed, in order to evaluate the tolerability of the compound by the mice at different concentrations (30, 45 and 60mM). The solutions were administered *iv* to C57B1/6 mice.

RESULTS

The ^{31}P-NMR and ^{1}H-NMR spectra, as well as the mass spectrum, were supporting the structure shown in Scheme 3. The *in vitro* test with mouse free hemoglobin from C57Bl/6 mice after incubation with compound 4 showed a P50 increase of 163%. Additionally, the P50 values in whole blood after incubation with compound 4 *in vitro* gave: a) for human blood a P50 increase of 27%; b) for pig blood a P50 increase of 27%; and c) for mouse blood a P50 increase of 20%. Finally, the *in vivo* injection of the compound at different concentrations gave: a) a P50 increase of 8.8% at 30 mM concentration; b) a P50 increase of 6.7% at 45mM concentration; c) a P50 increase of 10.2 and 14.8% at 60 mM concentration.

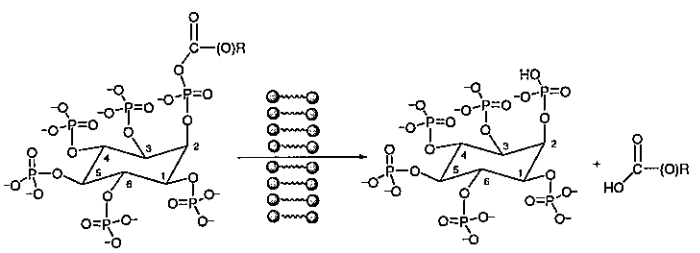


1: phytic acid **(IHP)**
(*myo*-Inositol Hexakis Phosphate)

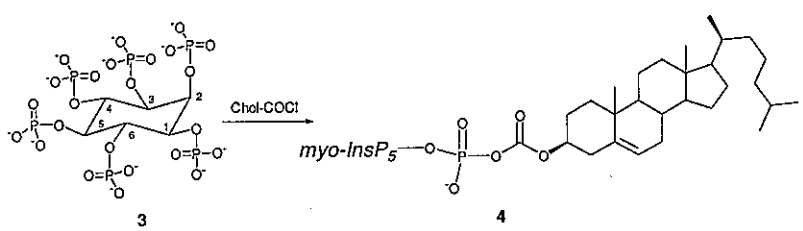


2: DPG
2,3-diphospho-*D*-glyceric acid

Scheme 1. Structures of IHP and DPG.



Scheme 2. The prodrug (covalent) approach. Transportation of IHP derivatives and hydrolysis of the linker inside the Red Blood Cells



3 4

Scheme 3. Synthesis of IHP-Cholesteryloxy carbonyl derivative

CONCLUSIONS

IHP-Chol compound caused a right shift in P50 of both, free hemoglobin and whole blood, respectively. *In vivo* administration of different concentrations of the compound all showed a right shift of the whole circulating blood of the injected mice, shift being between 7-15%. Compound 4 was non-toxic at these concentrations. It was well tolerated by mice. However, the number of animals used for the *in vivo* experiments was low. The experiments have to be repeated on a larger number of mice in order to evaluate tolerability and pharmacodynamic effects of the compound,

which might be a good candidate for development.

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