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Biopolymer strategy for the treatment of Wilson's disease

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ABSTRACT

Wilson's disease is a genetic disorder that causes excessive accumulation of copper in the body, leading to toxic damage, especially in the liver and nervous system. The current treatment cause burdensome side effects. We describe the use of chemically modified biopolymer carriers based on microcrystalline cellulose and chitosan containing the highly specific copper chelator 8-hydroxyquinoline as a new type of therapy for Wilson's disease. The chelators can scavenges copper ions released from food during digestion and copper ions present in secretions in the gastrointestinal tract. Because the chelator is covalently bound to indigestible biopolymer carriers (crosslinked chitosan or modified cellulose), it is not taken up by the gastrointestinal tract and it can be eliminated through the feces, avoiding unwanted side effects. This concept was tested on Wistar rats, which received a radioactive $^{64}CuCl_2$ solution together with the polymers with covalently bound 8-hydroxyquinoline through a gastric probe. ^{64}Copper complex uptake from the gastrointestinal tract was significantly inhibited by both chelating polymers. With the modified polymers, the presence of ^{64}Cu was detected mostly in the gastrointestinal tract, not in the internal organs. These findings indicate modified cellulose and crosslinked chitosan, with covalently bound 8-hydroxyquinoline exhibited the potential to be excellent therapeutics for treating Wilson's disease.

1. Introduction

Copper is an essential dietary nutrient needed for many important processes in the human body [1]. It is involved in numerous processes, and in particular, it is an essential constituent of enzymes, e.g., ferroxidases, lysyl oxidase, dopamine β-hydroxylase, copper/zinc superoxide dismutase, monoamine oxidase or tyrosinase [2]. Copper is also needed for mitochondrial respiration, melanin biosynthesis, iron homeostasis, connective tissue formation, dopamine metabolism and peptide amidation [3]. Defects in copper homeostasis can cause several human diseases, including Wilson's disease [4].

Wilson's disease is an autosomal recessive inherited disorder caused by a genetic mutation in the ATP7B gene [5]. ATP7B is responsible for copper transport into the *trans*-Golgi network and for the excretion of copper from the cell [6]. Mutations of ATP7B genes lead to high copper accumulation in the body, which causes oxidative-stress-related damage in the liver, brain and other parenchymal tissues (Fig. 1) [7].

The clinical manifestation of Wilson's disease mainly affects the liver and neurological system and can cause movement disorders, psychiatric disease or hemolytic anemia [8]. The most typical disorder is liver disease, which manifests as acute hepatitis or fulminant hepatic failure, chronic hepatitis and cirrhosis [3].

Currently, the standard treatment combines several approaches [9]. First, daily copper intake must be prevented by a lifelong diet with low copper contents. Therefore, the intake of nutrients with high copper contents must be avoided such as mushrooms, nuts or seafood. The average copper uptake for the normal human diet ranges from 0.5 mg to 2.5 mg per day [10]. However, a low copper diet is not sufficient by itself. Therefore, supplementing this diet with pharmacological treatment is also necessary.

The pharmacological treatment of Wilson's disease is a lifelong therapy based on low-molecular-weight copper-chelating agents e.g., penicillamine and trientine. These chelating agents increase copper elimination through the urine [3,11]. Another low-molecular-weight copper chelator used for the treatment is ammonium tetrathiomolybdate, which forms complexes with copper in the gastrointestinal tract (GIT). It also forms complexes with copper and albumin in the blood. Ammonium tetrathiomolybdate seems to be useful for

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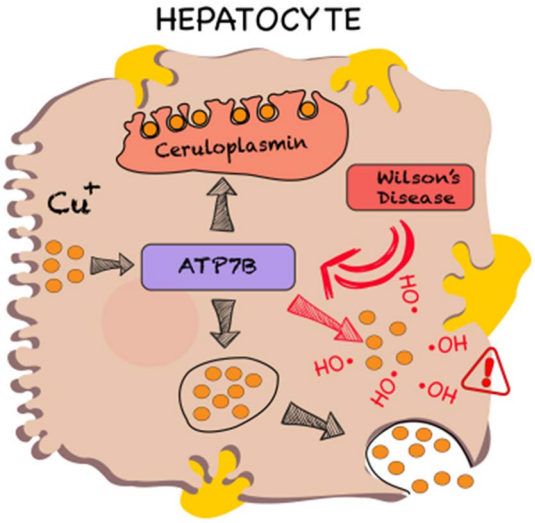

Fig. 1. The mechanism of copper accumulation in hepatocyte.

patients with neurological symptoms [12]. Along with the low-molecular-weight chelator therapy, high doses of divalent zinc salts can also be used as a maintenance therapy. Zinc (II) utilizes the same carriers for absorption in enterocytes as copper, and therefore, high amounts of zinc competitively decrease copper absorption [13].

Unfortunately, all these therapies have severe drawbacks. Even when patients follow a strict low-copper diet, completely avoiding copper intake is impossible. Main disadvantage of the pharmacological treatment is its presence in the other organs of the patients' body. Penicillamine has a high level of toxicity that causes severe side effects, e.g., immunologically induced lesions, pyridoxine deficiency or interference with collagen and elastin formation [3]. Zinc therapy is accompanied by strongly adverse gastrointestinal effects due to the high zinc uptake of 1200 mg/day, whereas normal daily intake of zinc is in the range 8 mg/day to 15 mg/day, i.e., a hundred times less than that of the therapy [14,15].

Copper enters the body with the food. Some copper is also secreted into the GIT with digestion fluids and further taken up by the GIT. The reuptake of copper from digestion fluids was reported to be even higher than the overall copper content taken up from food by the GIT [16]. Therefore, we focused on the GIT as the main place where copper (re) uptake occurs. The ideal copper scavenger should therefore be present only in the GIT and should not be able to enter the bloodstream or other inner organs of the body to avoid unwanted side effects. Such copper scavengers can thus be constructed using insoluble and indigestible polymer carriers for the chelators, which are in general non-resorbable and biologically neutral [17–20].

The polymer materials studied in this paper should be resistant to the action of pancreatic and faecal enzymes present in the GIT of human beings [21–23]. Microcrystalline cellulose is biologically non-digestible because the human body does not have the cellulolytic enzymes necessary to digest cellulose [24]. Modified celluloses such as ethylcellulose, carboxymethylcellulose, methylcellulose and hydroxymethylcellulose are also not digested by the colonic bacteria present in the GIT [25,26]. Moreover, microcrystalline cellulose is a common ingredient used in pharmaceutical industry and can also be found in many processed food products. The degree of digestion of different cellulose structures in rat models showed that microcrystalline cellulose was the least digestible of the tested materials [27]. However, different species are well known to have different digestion capabilities due to differences in the microflora of each organism.

The second examined biopolymer, crosslinked chitosan, was demonstrated not to undergo degradation by the action of colonic bacterial or pancreatic enzymes [22]. Crosslinked chitosan prevents subsequent degradation and dissolution in the stomach and fully allows the movement of the chitosan hydrogel through the GIT.

Both microcrystalline cellulose and crosslinked chitosan were modified by the covalently bound chelator 8-hydroxyquinoline (8HQ). The covalent bond prevents release the 8HQ into the body and its subsequent organ deposition. This chelator was chosen based on our previous studies [18]. We found that 8HQ is able to complex copper in wide range of pH. In vitro studies showed that Cu-8HQ complex is stable in wide range of pH and in presence of competing zinc ions favors Cu-8HQ complex over Zn. The abovementioned chelation properties of 8HQ are crucial because chelation occurs mostly in acidic pH (pH 2–4), but as the Cu-8HQ complex moves thru the GIT the pH rises to higher values. This pH shift can cause release of copper ions from Cu-8HQ complex. Moreover, in the same study we investigated Cu-8HQ complex chelation properties in the presence of free amino acids (stomach and small intestine model) that may lead to significant leakage of the absorbed copper form the sorbent. The intestinal environment was simulated by a solution of amino acids, with two of the most strongly copper chelating amino acids (l-cysteine and l-histidine) added at concentrations corresponding to their average daily uptake. The remaining amino acids were simulated with glycine at concentrations corresponding to amounts that are consistent with an average daily ingestion (50 g per 1.5 L).

We are describing completely new, advanced functional materials with suitable properties for the maintain treatment of Wilson's disease, which is a lifelong threatening disorder. This paper focuses on the development of next-generation, non-digestible, non-resorbable, biocompatible chelating polymers derived from microcrystalline cellulose and crosslinked chitosan particles for oral therapy for Wilson's disease. This therapy should not have the abovementioned drawbacks of the previous model systems, and it can potentially overcome all of the side effects exhibited by current therapies. The prepared materials contain a significantly higher content of chelating groups, and they do not accumulate in gastric mucosa, unlike our previous methacrylate system [28]. Our materials pass through the GIT with digested food. In our previous study we show and prove possess strong selective chelation abilities for copper in the biological environment model of used chelator 8HQ. In vivo studies on Wistar rats proved that copper scavengers based on cellulose and chitosan exhibit much higher copper chelating properties in vivo than the previously described materials. Our insoluble crosslinked systems with covalently bounded 8HQ should not be present in bloodstream or other organs. Therefore, it should not elicit side effects caused by organ deposition.

Our treatment is design as maintaining treatment lowering stress of the patients under current treatment. Application of prepared materials is shifting levels of copper that is uptaken from the food.

2. Experimental section

2.1. Materials and methods

The instrumental setup, chemical preparation and biodistribution information can all be found in the supplementary information. All animal experiments described hereinafter have been performed in accordance with the corresponding legislative, Act on Experimental Work with Animals (Decrees No. 311/97; 117/87 and Act No. 246/96 of the Czech Republic), which is fully compatible with the corresponding European Union directives.

3. Results and discussion

3.1. Polymer synthesis and characterization

3.1.1. Cellulose modification

Microcrystalline cellulose was modified via a series of reaction steps resulting in the final product 8HQ-cellulose (**4**) shown in Fig. 2(A).

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