Heme extract from horseradish peroxidase to use as nanomedicine for porphyria

Shiva Jafari*[1], Elham Bahreini[2] , Zahra Rikhtegaran Tehrani[3]

[1] Department of Immunology, Mazandaran University of Medical science, Sari, Iran

[2] Department of Biochemistry, Iran University of Medical Sciences, Tehran, Iran

[3] Department of Virology, Pasteur Institute of Iran, Tehran, Iran

***Corresponding author:** Shiva Jafari, Department of Immunology, Mazandaran University of Medical science, Sari, Iran. Email: Shivajaafari@gmail.com*

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ABSTRACT

Porphyria is a group of autosomal metabolic disorders which is characterized by genetic defects in heme biosynthesis pathway enzymes. The symptoms of the porphyria occur almost due to excess amount of heme precursors. Heme is a prosthetic group of hemoproteins including hemoglobin and essential enzymes such as peroxidase. It is illustrated that the heme molecule in horseradish peroxidase has a remarkable similarity to the one in human hemoglobin. In this hypothesis, we suggested that heme molecules extracted from horseradish peroxidase as a substitutional plant source, will be effective in heme deficiency disorders such as porphyria. So, heme derivatives could be regarded as a drug in order to regulate the excess heme production. It is also hypothesized that in order to maintain the heme molecules in an intact form and prevent from intestinal degradation, it could be conjugated with nanoparticles such as polylactic-*co*-glycolic acid (PLGA).

Keywords: Heme, horseradish peroxidase, nanomedicine, porphyria

INTRODUCTION

Porphyria is a group of autosomal metabolic disorders which is characterized by impairments in heme biosynthesis pathway [1]. Several types of porphyria have been identified by genetic cause and symptoms [2]. The prevalence of porphyria is probably ranges from 1 in 10,000 to 2 in 100, 000 individuals worldwide [3,4]. In most countries, porphyria cutanea tarda (PCT) and acute intermittent porphyria (AIP) are the most common type of porphyria. The most defected enzymes are uroporphyrinogen decarboxylase and porphobilinogen deaminase, respectively. The excess amount of heme precursors could lead to strange symptoms including photosensitivity, neuropsychiatric signs, abdominal pain, and anemia [5].

Heme, a complex of iron with protoporphyrin IX, is the oxygen-binding prosthetic group of hemoglobin and essential for cellular oxidation and reduction reactions. It is produced mainly in bone marrow [6]. Heme is also an essential component of other hemoproteins synthesized in liver hepatocytes, including cytochromes, catalase, and peroxidases [7].

Horseradish (armoracia rusticana) a perennial plant of the brassicaceae family, contains a peroxidase in its roots. Horseradish peroxidase plays a vital role in biomedical applications [8]. It is an alpha-helical protein which binds heme as a redox cofactor [9]. The heme group in horseradish peroxidase is very simpler than in mammalians which provides an excellent starting point in the study for a new drug design with applications for porphyria disease [10]. Peroxidases is studied due to the wide distribution among living organisms and the vital role in biomedical applications. Heme in high concentrations has negative inhibitory effect on producing the heme precursors and could balance the heme synthesis pathway. We believe that oral administration of heme-PLGA (polylactic-*co*-glycolic acid) would be preferable to intravenous delivery due to more benefits. It is expected that the heme molecule extracted from horseradish peroxidase will be effective in heme deficiency disorders.

The hypothesis

Control of intracellular heme levels was studied previously [11]. Former studies have proven that heme in high concentrations has negative inhibitory feedback on producing the heme precursors to balance the heme synthesis pathway [12]. This phenomenon

resulted in using heme derivatives as drug in acute porphyria to ameliorate the intensity of the attacks.

As it is illustrated in Figure 1, the heme molecule (B) from horseradish peroxidase has a remarkable similarity to the heme molecule (A) in human hemoglobin. Consequently, here it is hypothesized that using the heme molecule in horseradish peroxidase would be regarded as a drug in order to regulate the heme production pathway and reduce the overproduction of heme precursors including δ-aminolevulinic acid (ALA) which could cause the symptoms of the acute porphyria [9].

Natural heme which is the best source of iron for people who have iron deficiency, is easily absorbed by the body. Dietary heme is transported into intestinal mucosal cells, where it is mostly catabolized, with the subsequent appearance of inorganic iron in the portal vein [13]. In order to maintain the heme molecule in an intact form and prevent from intestinal degradation, it could be loaded in nanoparticles. Here we suggest that preparation of PLGA in conjugation with heme isolated from horseradish peroxidase would be administrated for oral delivery route. Heme-PLGA nanoparticles are considered to be long-circulating conjugates to improve drug stability and also lead to slowly drug release (Figure 2) [14].

Evaluation of hypothesis

This hypothesis would be assayed by administrating different dosage of extracted heme molecules conjugated to PLGA. Heme nanoparticles could be examined in rat model. Rats with true manifestations of AIP have increased urinary ALA and PBG levels during an attack. They could be checked for urinary ALA and PBG after nano-drug administration [15].

DISCUSSION

The only commercially available treatments are intravenous heme called panhematin (hemin for injection) and heme arginate (normosang) which are derived from processed red blood cells and the scientists are challenging with the stability which is increased and side effects which is decreased, such as potential transition of infections and possible contaminations during repeated injections [17].

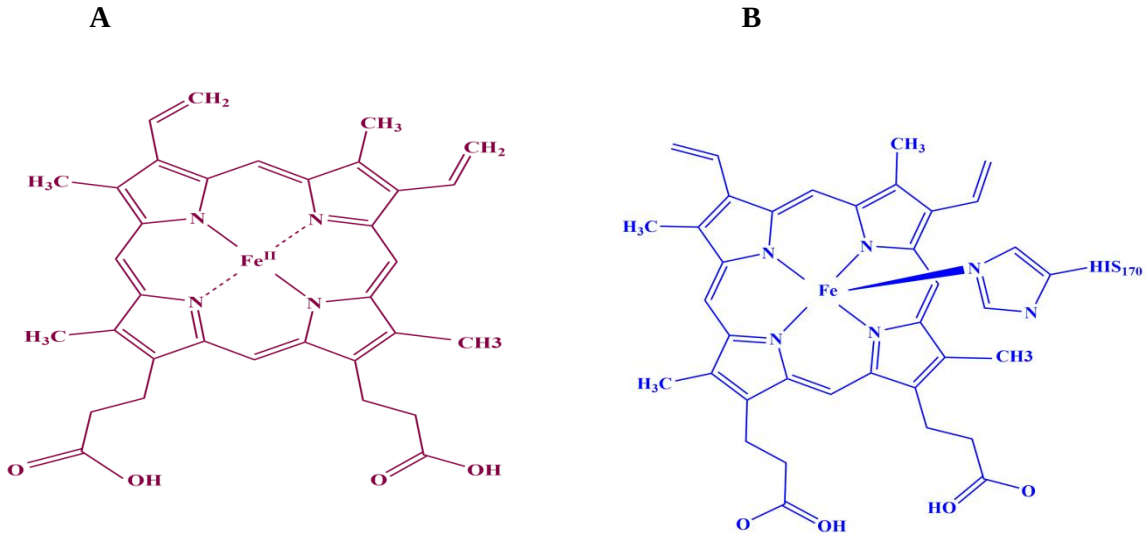

Figure 1. Comparison of two structures of heme; A: the natural type of heme present in human hemoglobin; B: the structure of heme in horseradish peroxidase.

We believe that oral administration could be preferable to intravenous delivery and offers more benefits. So, the heme extracted from plants like horseradish peroxidase, conjugated to PLGA could be less invasive and could be safer than common drugs.

Unlike to the most of important components which are transported in blood by carrier proteins, heme doesn't have the carrier protein in plasma. Free-heme enters the enterocyte by heme carrier protein-1 (HCP1) and broke down by heme oxygenase-1 in order to release containing iron. The released iron is used for cellular consumption including synthesis of heme in bone marrow or hemoprotein synthesis in liver [18]. It is hypothesized that nanoparticles could provide the opportunity to deliver the intact heme into the cells by endocytosis, without requiring carrier protein (Figure 2) [19].

Heme is well known as a negative feedback inhibitor of ALA in hepatocytes, the first committed precursor of tetrapyrrole biosynthesis [20]. ALA synthase is down regulated by heme concentration. The inhibition mechanism of ALA synthase by heme or hemin to decrease stability of mRNA synthesis and the intake of mRNA in the mitochondria [21]. Moreover, glucose and other metabolizable sugars also known to regulate the ALA synthase gene expression at the transcriptional level [5]. PLGA is a biodegradable carbohydrate polymer, which is metabolized in tricarboxylic acid cycle. Therefore, the process suggests that the combination of heme-PLGA could be an

optimized structure to inhibit the ALA synthase. On the other hand, it is known that excess intracellular free heme that is not associated with hemoproteins is potentially toxic to cells [22]. So, the administration dose of such heme-based drugs should be controlled by several experiments. Horseradish peroxidase is an enzyme belong to class III (classical secretory plant peroxidases) of the plant peroxidase superfamily [23]. Due to the increasing number of biomedical applications, numerous efforts have been made to purify the horseradish peroxidase from the root of the plant and to develop the methods of making recombinant horseradish peroxidase isoenzymes [24,25]. Also there have been successful reports of extraction methods in extracting heme molecules from hemoproteins and plants containing heme [26,27]. Hematin PLGA has also been developed to introduce nanoparticle for cancer targeting purpose recently [28].

CONCLUSION

This information suggests that the heme derived from horseradish peroxidase and its administration as heme-PLGA nanoparticle may foster the amelioration of porphyria by inhibitory feedback on ALA synthase. This may target the heme synthetic pathway in either erythroblasts or hepatocytes.

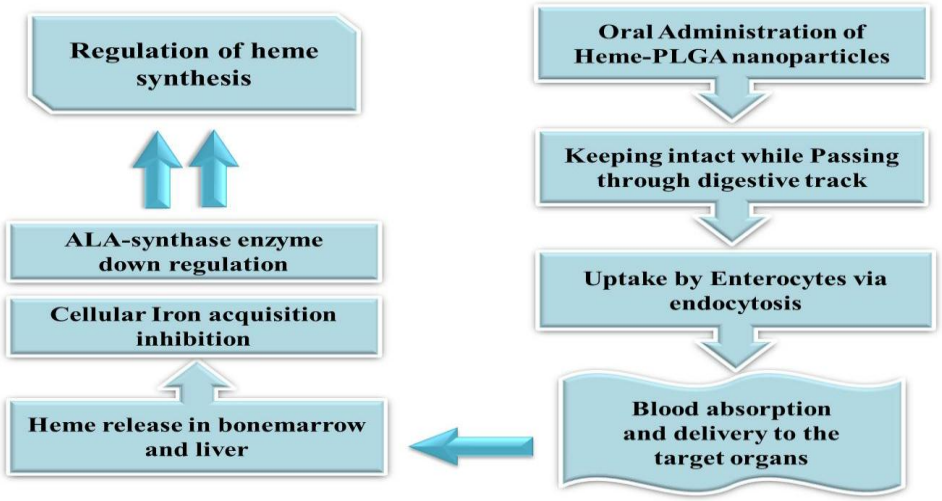

Figure 2. Absorption and cellular uptake of heme-PLGA nanoparticles, resulting in intact heme release and negative regulation of heme biosynthetic patheway in bone marrow and liver cells.

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