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5-Aminolevulinic acid regulates the inflammatory response and alloimmune reaction

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ABSTRACT

5-Aminolevulinic acid (5-ALA) is a naturally occurring amino acid and precursor of heme and protoporphyrin IX (PpIX). Exogenously administrated 5-ALA increases the accumulation of PpIX in tumor cells specifically due to the compromised metabolism of 5-ALA to heme in mitochondria. PpIX emits red fluorescence by the irradiation of blue light and the formation of reactive oxygen species and singlet oxygen. Thus, performing a photodynamic diagnosis (PDD) and photodynamic therapy (PDT) using 5-ALA have given rise to a new strategy for tumor diagnosis and therapy. In addition to the field of tumor therapy, 5-ALA has been implicated in the treatment of inflammatory disease, autoimmune disease and transplantation due to the anti-inflammation and immunoregulation properties that are elicited with the expression of heme oxygenase (HO)-1, an inducible enzyme that catalyzes the rate-limiting step in the oxidative degradation of heme to free iron, biliverdin and carbon monoxide (CO), in combination with sodium ferrous citrate (SFC), because an inhibitor of HO-1 abolishes the effects of 5-ALA. Furthermore, NF-E2-related factor 2 (Nrf2), mitogen-activated protein kinase (MAPK), and heme are involved in the HO-1 expression. Biliverdin and CO are also known to have anti-apoptotic, anti-inflammatory and immunoregulatory functions. We herein review the current use of 5-ALA in inflammatory diseases, transplantation medicine, and tumor therapy.

1. Introduction

5-Aminolevulinic acid (5-ALA) as described in detail in Table 1, a naturally occurring amino acid, is synthesized through the condensation of glycine and succinyl-CoA by the catalytic effect of 5-ALA synthase. In the cytosol, 5-ALA sequentially generates porphobilinogen, hydroxymethylbilane, uroporphyrinogen III and finally coproporphyrinogen III. In the mitochondrion, coproporphyrinogen III is metabolized to coproporphyrinogen III, protoporphyrinogen IX and protoporphyrin IX, into which iron is inserted via a ferrochelatase-catalyzed reaction, the latter resulting in the formation of heme [1–3]. 5-ALA is utilized as a precursor of a photosensitizer for performing a photodynamic diagnosis (PDD) and photodynamic therapy (PDT) to confirm and kill tumor cells [1,4]. Due to the substance's short half-life, the toxicity of 5-ALA is very low. The half-life of 5-ALA by 5-ALA administration (20 mg/kg) is 55.2 min in human (our unpublished data), 45 min in human (100 mg) [5], and 40.7 min in dog (7.29 mg/kg) [6]. As a result, clinically relevant photosensitivity is negligible. In addition to the utilization of PDD and PDT, 5-ALA has been undertaken to treat inflammatory disease, autoimmune disease and transplantation due to the anti-inflammation and immunoregulation properties by upregulation of heme oxygenase (HO)-1 expression and release of heme metabolites.

2. Tumor therapy

2.1. Tumor cells and PpIX accumulation

Several factors, although the preferential mechanisms are unknown, have been demonstrated to be involved in the accumulation of PpIX. The expression level of peptide transporter 1 (PEPT1)/solute carrier family 15 member 1 (SLC15A1), ATP-binding cassette sub-family G member 2 (ABCG2) [7,8], SLC6A6 and SLC6A13 [9], which are responsible for the import of 5-ALA and export of PpIX, and the activity of ferrochelatase [10,11] are key regulators of PpIX accumulation. Heme biosynthesis and the iron metabolism are also involved in PpIX accumulation. Inner mitochondrial membrane proteins (mitoferrin-1, which is mainly expressed in erythroid cells, and mitoferrin-2, which is expressed in various cells) are dependent on the transport of iron into mitochondria [12]. In the last step of heme biosynthesis, frataxin-mediated iron delivery to ferrochelatase occurs [13]. Ohgari et al. showed that the overexpression of mitoferrin-2 decreased PpIX accumulation [14]. Furthermore, Sawamoto et al. showed that frataxin overexpressing cells had decreased the amount of intracellular PpIX accumulation [15].

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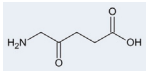
Table 1
Property of 5-aminolevulinic acid hydrochloride.

Structural formula	$C_5H_9NO_3 \cdot HCl$ 
Synonym	5-Amino-4-oxopentanoic acid hydrochloride
CAS no.	No. 5451–09-2
Molecular weight	167.6
Color	White to off-white
Melting point (°C)	144–147 (degradation)
pH (1 mol/L)	1.7
Water solubility	Very soluble

2.2. 5-ALA and PDD

As shown in Fig. 1 (left), 5-ALA is incorporated into cells by exogenous administration and converted into PpIX, which is a fluorescent and has photoactivity. Specific irradiation at 375–475 nm wavelength excites PpIX and generates red fluorescence. PpIX has another property, which is its accumulation in tumor cells, and augmented red fluorescence results in the identification of tumor cells. Its emission of red fluorescence and accumulation in tumor cells are fundamental for PDD [16–18].

2.3. 5-ALA and PDT

PDT has become one of the standard procedures of cancer therapy, which is achieved by the generation of reactive oxygen species (ROS) and singlet oxygen by porphyrin derivatives through the irradiation of visible light [19–21]. PpIX metabolized from 5-ALA is one of the most efficacious photosensitizing agents for PDT [22,23]. The tumor-preferential accumulation of PpIX allows tumor therapy by 5-ALA-mediated PDT (Fig. 1 left).

3. 5-ALA and anti-inflammatory and immunoregulatory properties

3.1. HO-1

5-ALA has been demonstrated to induce the upregulation of heme oxygenase (HO)-1 mRNA levels [24,25]. In heme catabolism, HO functions as a rate-limiting enzyme. As shown in Fig. 1 (right), HO catalyzes heme degradation, thereby producing biliverdin, carbon monoxide (CO) and iron. HO-1 is the inducible isoform of HO [26] and the expression of HO-1 is induced by a range of stress stimuli [27–34], including heme [30,35] and other metalloporphyrins [36,37], in a number of cell types. Endotoxin exposure to mice with HO-1 deficiency results in a higher mortality from endotoxic shock, the upregulation of splenic proinflammatory cytokine secretion and increased hepatocellular necrosis compared with wild-type mice [38]. A study on individuals with HO-1-deficiency has strengthened the above finding and HO-1 has been shown to act as an important cytoprotective factor in counteracting the detrimental increase in oxidative injury and inflammation [39]. Similarly, an in vitro study confirmed that there is a reduction of stress resistance in HO-1-deficient cells [40].

3.2. HO-1 and signal transduction pathway

The signaling mechanisms that activate the transcription of HO-1 remain insufficiently elucidated. Many studies have focused on the activation of mitogen-activated protein kinases (MAPKs) related to cell growth and the stress response. The pathway of p38, c-Jun. N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK) appear to participate to some degree in the upregulation of the HO-1 expression in response to various stimuli [41–43]. MAPK signaling leads to the translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) to the nucleus [43]. Nrf2 is a basic leucine zipper (bZip) transcription factor [44]. Under basal conditions, Bach1, which is also a bZip transcription factor [45], forms heterodimers with musculoaponeurotic fibrosarcoma oncogene homolog K (MafK), and these heterodimers

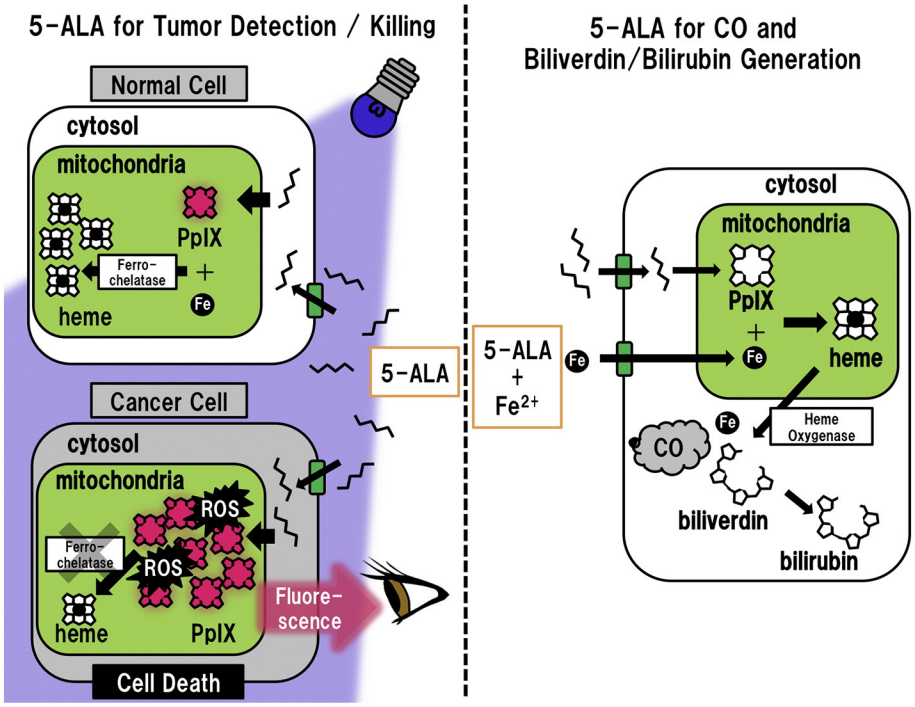


Fig. 1. PDD, PDT and anti-inflammatory/immunoregulatory structure and property of 5-ALA. In normal cell, 5-ALA produces PpIX, then heme is produced by aiding in the insertion of iron by ferrochelatase. In contrast, less ferrochelatase expression in cancer cell gives rise to accumulation of PpIX. 5-ALA with SFC produces heme and upregulates the expression of HO-1, which catalyzes heme to CO, Fe and biliverdin.

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