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Review

Aminolevulinic Acid (ALA) as a Prodrug in Photodynamic Therapy of Cancer

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Abstract: Aminolevulinic acid (ALA) is an endogenous metabolite normally formed in the mitochondria from succinyl-CoA and glycine. Conjugation of eight ALA molecules yields protoporphyrin IX (PpIX) and finally leads to formation of heme. Conversion of PpIX to its downstream substrates requires the activity of a rate-limiting enzyme ferrochelatase. When ALA is administered externally the abundantly produced PpIX cannot be quickly converted to its final product - heme by ferrochelatase and therefore accumulates within cells. Since PpIX is a potent photosensitizer this metabolic pathway can be exploited in photodynamic therapy (PDT). This is an already approved therapeutic strategy making ALA one of the most successful prodrugs used in cancer treatment.

Key words: 5-aminolevulinic acid; photodynamic therapy; cancer; laser; singlet oxygen

1. Introduction

Photodynamic therapy (PDT) is a minimally invasive therapeutic modality used in the management of various cancerous and pre-malignant diseases. It

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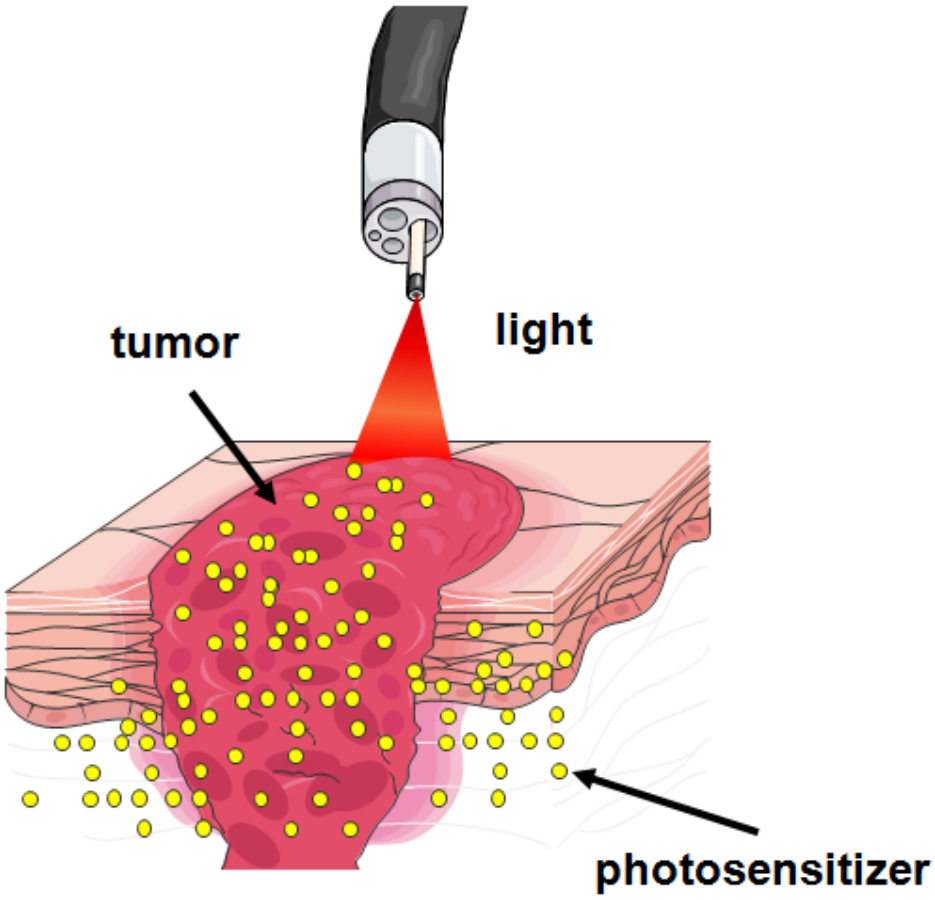
1. Introduction

Photodynamic therapy (PDT) is a minimally invasive therapeutic modality used in the management of various cancerous and pre-malignant diseases. It involves the systemic administration of a non-toxic photosensitizing (PS) drug, which accumulates in host and tumor cells, and subsequent illumination of

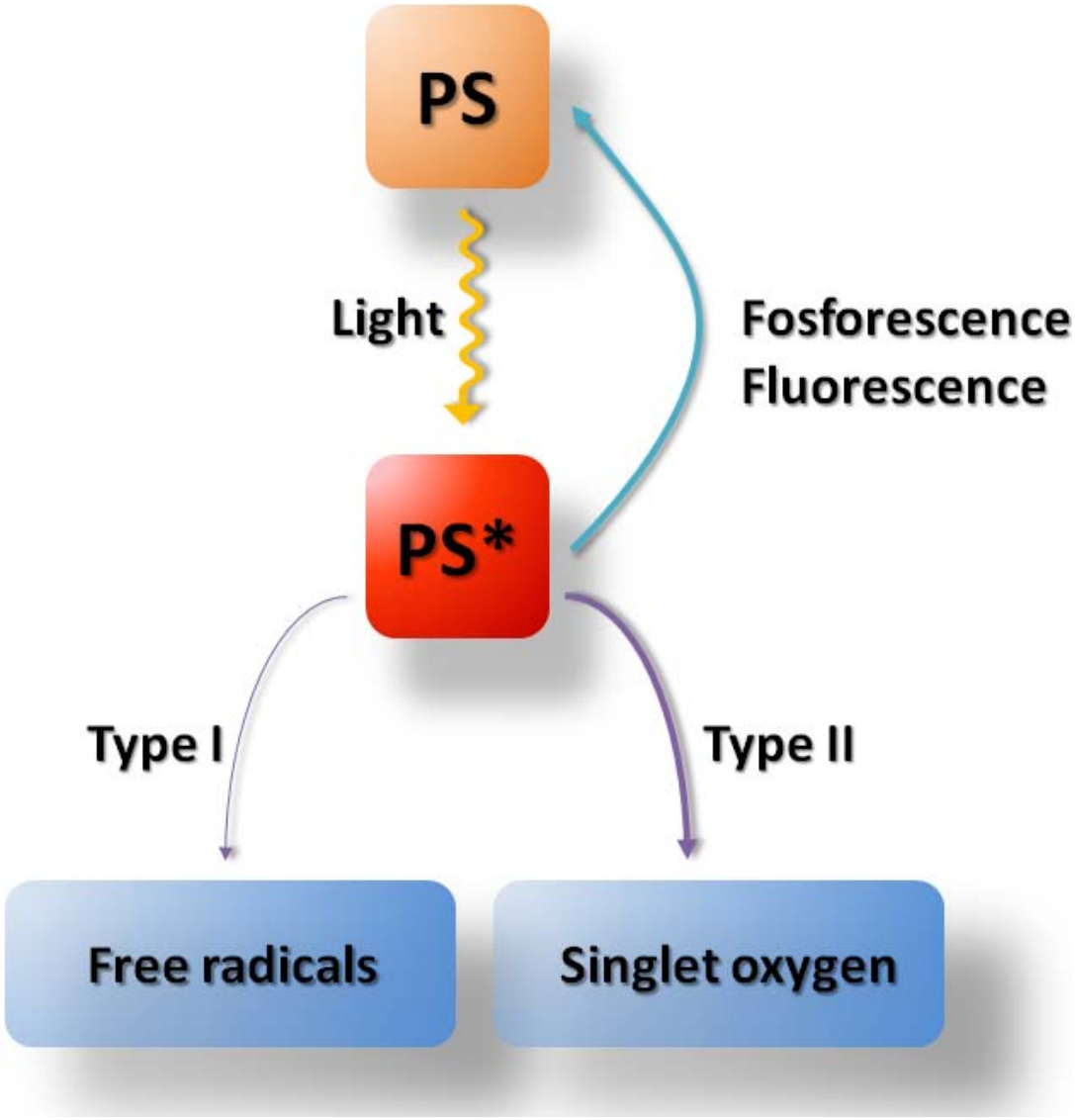
the tumor site with visible light, corresponding to the appropriate photosensitizer absorption wavelength (Figure 1).

Figure 1. Overview of PDT. Following photosensitizer administration it undergoes systemic distribution and selectively accumulates in the tumor. Illumination activates the photosensitizer and in the presence of molecular oxygen triggers a photochemical reaction that culminates in the production of $^1O_2$.



The excited photosensitizer contributes to the generation of singlet oxygen and other reactive oxygen species (Figure 2), which results in the oxidative damage to intracellular macromolecules and consequently leads to cell death. The mode of PDT-induced cell death is usually a mixture of apoptosis, necrosis and autophagy, with the dominance of a particular process depending on the PS (mainly its subcellular localization) as well as light fluence. It is generally agreed that apart from the direct cellular cytotoxicity, two other important factors contribute to the overall PDT effect: the vascular shutdown and local inflammatory reaction [1-4]. One of the major advantages of PDT over other anticancer treatment modalities is its high degree of selectivity. This is accomplished via the combination of two inactive components, visible light and a photosensitizing drug, which applied together in the presence of oxygen lead to generation of cytotoxic intermediates that effectively kill tumor cells [5].

Figure 2. Types of oxidative reactions during PDT. Light with sufficient energy and wavelength matching absorption spectrum of the photosensitizer (PS) can activate a photochemical reaction that leads to formation of activated PS molecule (denoted by asterisk). Activated PS can lose its energy by emitting of visible light (fosforescence or fluorescence). Alternatively it may generate singlet oxygen in a type II reaction or free radicals in a type I reaction.



As the PS alone is non-toxic and ineffective, to some extent it can be considered as a prodrug. However, additional selectivity of PDT may be achieved by the administration of a PS precursor. The only clinically approved example of such a compound is δ-aminolevulinic acid (ALA), a precursor of the natural photosensitizer phrotoporphyrin IX (PpIX). In contrast to exogenously administered PSs such as Photofrin, the photodynamically inactive, non-selective and non-toxic compound, ALA, is intracellularly metabolized to the photodynamically active PpIX. Subsequent illumination of the tumor site with red light activates PpIX, triggers the oxidative damage and induces cytotoxicity [6].

ALA is a naturally occurring compound, the early intermediate in the heme biosynthesis pathway. For therapeutic purposes ALA is administered topically or systemically and penetrates non-selectively into all cells, where it is metabolized to an active sensitizer PpIX. The bioactivation of ALA utilizes the enzyme machinery of the heme biosynthesis pathway. Although nearly all human cell types express the enzymes involved in the heme synthesis, a distinct activity of the enzymes in tumor as compared with normal cells leads to a higher PpIX accumulation within transformed cells [7].

For the last two decades a substantial amount of research has been focused on the elucidation of the mechanism of ALA-PDT and the improvement of its therapeutic activity. ALA is a polar molecule and in physiological pH occurs mainly as a charged zwitterion, which accounts for its low lipid solubility and reduced bioavailability. Further modifications of ALA aimed at improving its cellular permeability, increased stability in physiologic pH, increased selectivity and limitation of side effects, are important challenges in order to extend the clinical use of ALA-PDT. Since the very first topical application of ALA in the treatment of basal cell carcinoma in 1990 [8], the clinical use of ALA-PDT is still growing. Nowadays, PDT with ALA and its esters is an approved treatment of several malignant and premalignant conditions such as actinic keratosis, basal cell carcinoma, Bowen's disease, bladder cancer and others. This review presents the use of ALA and its derivatives as prodrugs in PDT and summarizes the preclinical and clinical results of the treatment.

2. Metabolism of ALA

2.1. Heme Biosynthesis

The synthesis of ALA is the first and rate-limiting step in the biosynthesis of heme [9-11]. ALA is normally synthesized in mitochondria in the condensation reaction between glycine and succinyl-CoA (Figure 3). The reaction is catalyzed by ALA synthase (ALAS) and requires pyridoxal-5-phosphate (PLP) as a cofactor. In mammals, two isoforms of ALA synthase have been identified: ALAS1, which is a housekeeping enzyme and ALAS2, which is expressed only in erythroid precursors [12].

After being synthesized ALA reaches cytosol, where it undergoes a condensation reaction. The reaction occurs between two ALA molecules with the aid of zinc-dependent enzyme – aminolevulinate dehydratase (ALAD) – and leads to the formation of porphobilinogen (PBG). ALAD, also known as porphobilinogen synthase (PBGS) comprises four homodimers, each of them having one active site [13]. Two molecules of ALA bind non-symmetrically to the active site, finally leading to the synthesis of PBG [14].

The next step in heme biosynthesis involves combining four molecules of PBG to form an unstable tetrapyrolle - hydroxymethylbilane (HMB). The reaction is catalyzed by porphobilinogen deaminase (PBDG), an enzyme containing dipyrromethane in its active site. Dipyrromethane is a co-factor covalently bound to the enzyme and consists of two PBG molecules. Four additional molecules of PBG attach to dipyrromethane leading to the formation of hexapyrolle. Afterwards, in the hydrolytic reaction, cleavage of the distal tetrapyrolle occurs, resulting in the release of HMB [15]. Hydroxymethylbilane can then enter two pathways. The first one uses uroporphyrinogen III synthase (URO3S) to close the HMB macrocycle leading to conversion of tetrapyrolle to uroporphyrinogen III. Alternatively, HMB can undergo spontaneous cyclization, which leads to the formation of

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