Review

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Pros, cons and future prospects of ALA-photodiagnosis, phototherapy and pharmacology in cancer therapy – A mini review

Abstract: 5-Aminolevulinic acid (ALA)-induced photodynamic therapy (ALA-PDT) has achieved remarkable research accomplishments over the past 30 years, yet its application in medical oncology still awaits clear recognition as a valid alternative therapeutic modality. It is well documented that topical ALA-PDT enables the treatment of multiple skin lesions simultaneously, and provides excellent cosmetic results with no acquired multi-drug resistance (MDR). Furthermore, upon disease recurrence the treatment can be repeated resulting in the same therapeutic efficacy. Additionally, in oncological surgery, ALA fluorescence-guided resection is a practical and simple method for visualizing intra-operative brain and urological tumors with millimeter accuracy. The urgent challenge is to direct future research of ALA-phototherapy and fluorescence diagnosis to the maturation of their medical status in oncology. Therefore, the future objectives are to amplify critical evidence-based results of ALA-PDT safety and efficacy and to validate its unique advantages over other technologies. Strong statistical PDT documentation and the positive predictive values of protoporphyrin IX (PpIX)-guided surgery will persuade the medical community to implement ALA-based therapeutics into standard clinical and surgical oncology practice. Research must address the phenomenon that no MDR develops as a consequence of PDT, since MDR is the major stumbling block in oncological therapeutics. A feasible goal should be to improve ALA administration protocols based on recent knowledge that preactivation of the enzyme porphobilinogen deaminase enhances PpIX accumulation in cancer cells and photodestruction. Moreover the recent introduction of multifunctional ALA prodrugs that maximize photosensitizer biosynthesis, targeting multiple subcellular targets, may increase PDT anti-cancer efficacy in additional disease settings. In conclusion, well-documented clinical results, new ALA delivery protocols, and novel multifunctional ALA prodrugs may advance ALA-PDT to becoming a front-line cancer therapy.

Keywords: ALA; PDT; multi-drug resistance; prodrugs; ALA-stimulated fluorescence image-guidance.


Schlüsselwörter: ALA; PDT; Mehrfachresistenzen; Medikamentenvorstufen; ALA-stimulierte Fluoreszenzbildgebung.

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1 Introduction

1.1 Basic principle of phototherapy

Photodynamic therapy (PDT) of cancer is based on the interaction of three components, (i) a photosensitizer, (ii) light and (iii) oxygen, to induce tumor death by necrosis and apoptosis. The photosensitizer itself is essentially nontoxic and is rapidly metabolized or excreted from the body; when it absorbs light it transfers energy to oxygen present in the aqueous milieu, producing singlet oxygen ($^{1}\text{O}_2$), a high-energy, toxic reactive oxygen species (ROS). Singlet oxygen molecules rapidly move through the cell milieu potently inducing free radicals, which oxidize cellular macromolecules (proteins, lipids, DNA) cumulatively resulting in cytotoxicity. The selectivity of PDT is dependant on the accumulation of an exogenous or endogenous photosensitizer in the tumor, including its vasculature. The efficacy of PDT is restricted by the degree of light penetration and oxygen availability in the target tissue, which are obligatory for photodestruction of the tumor [1].

1.2 Specific role of protoporphyrin IX in ALA-induced PDT

“Cancer cells produce excess protoporphyrin”. This quote was a basic conclusion in one of the very first publications describing the concept of 5-aminolevulinic acid (ALA)-induced PDT (ALA-PDT) [2] and followed by “Photo-activated endogenous porphyrins kill cancer cells of different origins” [3]. ALA-induced PDT is based on self-supplying protoporphyrin IX (PpIX), a powerful photosensitizer, as the final metabolite of neoplastic cells, due to partial upregulation of the heme synthesis pathway metabolizing ALA into PpIX [4]. PpIX levels selectively increase in tumors compared with the non-malignant surrounding tissue. The synthesis of PpIX and its accumulation within transformed pre-malignant and malignant cells is time-dependent and selective, with an accumulation ratio which is at least 3 times higher in these cells than in the surrounding normal tissue. ALA dehydratase, the first cytosolic enzyme, condenses two ALA molecules to form a pyrrole ring compound, porphobilinogen, which is the substrate of the rate-limiting enzyme, porphobilinogen deaminase (PBG-D). The main product synthesized by PBG-D is hydromethylbilane in a process that depends on the self-activation of the enzyme by prior production of dipyromethane, the internal cofactor of the enzyme. The porphyrin ring is then closed and finally step-by-step, PpIX is produced. As a general rule PpIX is a major product of the heme synthesis pathway in neoplastic cells, due to low activity of mitochondrial ferrochelatase which inserts iron to produce heme [5].

These experimental results are the foundation of ALA-stimulated phototherapy and photodagnosis proven by hundreds of studies but they still lack the molecular basis to explain why ALA is, as a rule, taken up preferentially by neoplastic cells, although cancer is a heterogeneous disease [6, 7].

In dermatology, ALA-PDT is mainly used in the treatment of superficial skin cancers: actinic keratoses, Bowen’s disease and superficial basal cell carcinomas (BCCs). Clinical studies have revealed that PDT can be curative, particularly in early stage tumors. Hence, the advantages of topical ALA-PDT are: ability to treat multiple lesions simultaneously, low invasiveness of therapy, good tolerance and excellent cosmetic results and most importantly, no acquired multi-drug resistance (MDR) to ALA-PDT has been reported and therefore the treatment can be repeated with the same efficacy [8–12]. The main
concern in chemotherapeutic cancer therapy is the significant number of drug-resistant cells which remain even after most of the drug-sensitive cells have been eliminated. With each round of chemotherapy, successive, alternative chemotherapies become more likely to fail since the remaining tumor cells develop resistance even to structurally and mechanistically unrelated drugs and as a consequence; treatment options become more limited [13, 14].

Another advantage of endogenous PpIX, when compared to exogenous photosensitizers, is its rapid metabolism which significantly reduces the period of cutaneous photosensitivity and significantly improves the quality of life. Moreover, it shows low toxicity in normal tissue, with only minor systemic effects, making ALA-PDT a valuable therapeutic modality in light penetrable or fiber-transduced tissues [15].

Most important, PpIX is a remarkable tool in surgery where it plays a diagnostic role in the form of red fluorescence signal for demarcation of cancer tissues during excision. Fluorescence-guided resection, stimulated by ALA pre-medication, is a practical and simple tool for the neurosurgeon that provides a straightforward way to visualize intra-operative malignant glioma [16–18]. It has been noted that PpIX bleaching in the intra-operative setting occurs only after more than 1 h of continuous white-light illumination (depending on the illumination intensity), which is ample time for this type of surgery. The difference in outcome between PDT and PpIX fluorescence in these two different cancer settings (therapy and surgical diagnosis) is the energy of the illuminating light source, i.e., the excitation and emission spectra, and the light intensities. Low energy light (excitation at 415 nm; emission at 630 nm) is useful for fluorescence while high energy light (excitation at 415 or 630 nm) activates singlet oxygen production (Figure 1). Used light fluences for PDT or fluorescence diagnostics may vary between different set-ups.

A plausible explanation for the phenomena of preferential synthesis and accumulation of PpIX in cancer tissues may lie in the metabolic transformation that occurs in the final stages of carcinogenesis. Figure 2 depicts central cancer cells adaptations that are essential in malignant conversion and tumor progression including genetic and epigenetic modifications, alterations in cell death pathway activation, resistance to drugs and most importantly anaerobic metabolism [19, 20]. Mitochondrial aerobic adenosine triphosphate (ATP) synthesis is largely dependent on heme supply through the heme synthesis pathway where heme is crucial as the active site cytochromes through electron transport chain reactions [21, 22]. The unique anaerobic metabolic phenotype of cancer cells promotes altered regulation of the heme synthesis pathway stimulated by the ALA exogenous supply. Exogenous ALA supply circumvents the first mitochondrial synthesis by enzyme ALA synthase which is persistently down-regulated in neoplastic cells. Hence ALA is metabolized by the second enzyme of the pathway, the cytosolic ALA dehydratase, followed by PBG-D to produce tetrapyrroles. The last tetrapyrrole PpIX is imported into the mitochondria where ferrochelatase, inserts Fe$^{2+}$ into PpIX to produce heme. Cancer cells generally possess low activity of ferrochelatase so PpIX is accumulated as a consequence in the mitochondria as part of the metabolic cancer syndrome [5, 23, 24].

2 Current status of ALA phototherapy and photodiagnosis

The multiple attributes of ALA-stimulated PDT provide a broad range of advantages in cancer therapy:
it can be used for pre-malignant and malignant diseases,
– it can precisely target a tumor and adjacent metastases,
– it has no long-term side effects,
– it gives excellent aesthetical results,
– it can be used as an adjuvant to surgery,
– it can be repeated several times at the same site with similar efficacies and
– ALA can be applied topically, orally or by instillation or inhalation.

ALA-PDT represents an alternative treatment option to the existing range of cancer therapies and can be used in conjugation with them (Figure 3). The most common medical use of ALA-PDT is in dermatology for therapy of actinic keratoses, Bowen’s disease, superficial BCCs and in certain thin nodular BCCs, with a superior cosmetic outcome compared to conventional therapies matched with high anti-cancer efficacy [25].

ALA-stimulated fluorescence image-guidance (AFG) during surgery of malignant lesions is a major clinical methodology, particularly in resection of gliomas. There is strong medical evidence that AFG use in surgery increases diagnostic accuracy and extends tumor resection-enhancing quality of life, thus prolonging survival in patients with high-grade malignant gliomas, pediatric brain tumors, spinal tumors and early redo surgery for glioblastoma [16, 26–29]. Macroscopic fluorescence qualities predict solid and infiltrating tumor, providing useful information during resection. Stummer et al. [18] conclude that ALA-derived fluorescence appears to be a superior method for contrast enhancement on magnetic resonance imaging for demarcation of residual glioma tumors with millimeter resolution, thus offering surgeons a precise guidance during resection.

By contrast to AFG use in glioma surgery, a clinical study of AFG in urology surgery of non-muscle invasive bladder cancer did not demonstrate a clear advantage over existing imaging techniques, since there remain doubts about whether it improves tumor detection or reduces residual disease after transurethral resection of bladder tumor compared with white-light cystoscopy [30, 31]. There is statistical evidence that AFG improves...
recurrence-free survival but not disease progression, indicating the limitations of AFG and highlighting its cost effectiveness, since its use results in elevated surgery costs. Thus, in urology further clinical research is needed to evaluate the cost effectiveness of AFG cystoscopy, leaving open the question and implied challenges raised by Cordeiro et al. [30]: “Is photodynamic diagnosis ready for introduction into urological clinical practice?” [31].

Although remarkable, the clinical achievements due to the introduction of ALA in PDT and AFG surgery have not yet justified their use as a method of choice in oncology. The obvious remedy for this situation is further clinical work and industrial investment for evaluating the cost effectiveness of these methods. In the meantime, however, as a result of the poor implementation of ALA-PDT and AFG in clinical practice, regulatory objections restrict the use of ALA in the United States, and as such clinical research in this field remains limited [32–34].

3 Obstacles for the clinical acceptance of phototherapy and photodiagnosis

The main problem with ALA in clinical use is regulatory. It has not been proven to improve overall outcome as a diagnostic marker of tumor tissue in surgery in advanced clinical trials. Furthermore, efficacy in comparison to other methodologists still must be proved in controlled clinical studies while balancing their feasibility in the current socioeconomic milieu.

Regulatory restrictions on ALA use in the United States is limited for use only in centers with limited protocols, whereas other centers are excluded which prevents further clinical research. Regulatory bodies now stipulate that the use of AFG in surgery must be shown to improve the cancer patient’s outcome rather than to provide tumor demarcation during surgery. In response to this requirement, Stummer [34] questioned whether the scientific community is really able to study specificity and sensitivity of AFG in conjunction with intra-operative methods for glioblastoma patients. Based on his medical experience his answer is “no”, adding his phrase “I want to reassure the authors that any fear of 5-ALA is not justified”. Stummer urges that, based on his surgical experience, the correct measure of AFG clinical value in glioblastoma surgery would be in its positive predictive value as a specific fluorescence marker of tumor tissue [18], rather than on its ability to improve patient survival statistics.

Therefore, ALA clinical development appears associated with a high clinical and financial risk, which has most likely deterred major investors and Pharma companies from rising to the ALA challenge. What scientific and medical advances can be achieved that will assuage sceptics of their reticence, and justify renewed investment in ALA applications in anti-cancer therapy?

4 Future challenges in phototherapy and photodiagnosis

4.1 The “fear of PDT?”

Despite the large volume of published experimental and clinical results in the ALA fields, these therapeutic modalities have not become methods of choice in oncology. The main reason appears to be insufficient adequately randomized controlled trials and evidence-based statistics. Such research involves large investments by pharmaceutical companies in order to achieve a critical mass of convincing clinical results that these methods have clear advantages over existing treatment options. Thus researchers need to engage pharmaceutical companies to invest in ALA research to achieve gradual “ALA” illumination in the eyes of these key players. In the PDT field, it is easy to get the impression that the conservative medical community has developed an innate fear of PDT because it combines both biochemical and biophysical approaches [34].

4.2 Multi-drug resistance post ALA-PDT

The advantages of PDT in overcoming MDR should be further supported by clinical evidence-based data. Most neoplastic cells develop secondary resistance to chemotherapy, which remains the major obstacle to cancer treatment. With each round of chemotherapy, any successive, alternative chemotherapies become more likely to fail since the remaining tumor cells develop resistance making treatment options more limited. The Achilles’ heel of chemotherapy and radiotherapy is the development of MDR and therefore, cancer cell survive high intracellular concentrations of anti-tumor agents and cytotoxic effects. Conversely, ALA-PDT impairs the
tumor chemo-resistant properties of head and neck cancer-derived stem cells [35]. There is strong evidence that post ALA-PDT, treatment can be repeated with the same efficacy each time yet this effect should be further studied, both experimentally and clinically. The possible combined modalities of ALA-PDT and chemotherapy during solid tumor management is of particular interest [36]. The mechanism of ALA-PDT cytotoxicity is toxic levels of ROS fed by elevated heme synthesis pathway activity. Resistance is unlikely to appear through improved quenching of ROS, rather through mutations or epigenetic reduction of heme pathway flux. However, here the cancer cell may be dependent upon this pathway for its survival. Most importantly, the tumor vasculature endothelial cells are rapidly and lethally damaged by PDT, and these cells do not have the advantage of genetic and epigenetic heterogeneity as a pool of diversity for evolving PDT resistance.

4.3 Develop ALA prodrugs!

Multifunctional acyloxyalkyl ester prodrugs induce efficient PpIX synthesis due to up-regulated PpIX biosynthesis and efficient photodynamic killing of cancer cells. One of these prodrugs, AlaAcBu, releases three active products: ALA, acetaldehyde and butyric acid. They stimulate independent pathways through activation of specific biochemical routes. ALA stimulates PpIX synthesis and PDT, acetaldehyde endorses dark tumor cytotoxicity and butyric acid inhibits histone deacetylase, leading to gene expression and tumor differentiation. All are targeted to boost anticancer actions and to reduce tumor recurrence [36–38].

4.4 Improve ALA administration protocols!

In our latest research, we investigated the effect of ALA pre-treatment on PBG-D expression and activity [39]. ALA incubation for 24 h activated PBG-D activity by 250% in comparison to control, with no significant difference in the PBG-D expression, thus the PpIX accelerated synthesis was due to PBG-D activation alone. We also developed a protocol for boosting PpIX synthesis in MDR-cells by two rounds of ALA exposure [unpublished data]: The first induces synthesis of dipyrromethane from ALA, which is essential for PBG-D activity, while the second provides the precursor for PpIX synthesis and photodynamic cell killing. Accordingly, pre-activation of the enzyme PBG-D both enhances PpIX accumulation in cancer cells and photo-destruction.

4.5 “Must” applications for PpIX-guided tumor surgery

While ALA-induced PpIX fluorescence offers the surgeon millimeter resolution during glioblastoma resection, other surgical oncology fields may also benefit from this technique. Clinical research is needed to identify additional specialized areas of surgical oncology where AFG is urgently needed to increases the radicalism of the surgical procedure that may give rise to improved progression-free survival. One urgent “must” application during redo surgery of residual tumor of glioblastoma regrowth is the combination of tumor resection with ALA-PDT [28]. We further propose another application for ALA-derived AFG for fibromatosis surgery which may lead to the improved management of benign soft tissue tumors.

5 Conclusion

In conclusion, new ALA delivery protocols and novel generation of multifunctional ALA prodrugs may render ALA-PDT a more potent method for front-line cancer therapy. It is highly likely that the future of ALA-PDT will involve the introduction of combinatory concepts of multifunctional ALA prodrugs to maximize sensitizer biosynthesis and to hit tumors in multiple sub-cellular targets that are independent of MDR mechanisms.

References


