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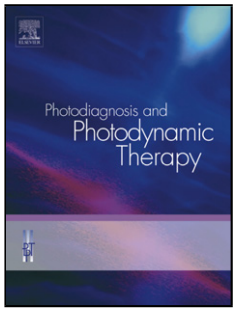

Photodynamic therapy for atherosclerosis. The potential of Indocyanine Green

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Photodynamic therapy for atherosclerosis. The potential of Indocyanine Green.

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Highlights

- Prevention and treatment of restenosis in cardiovascular disease is an important problem.
- Cardiovascular applications of photodynamic therapy are evaluated.
- The ideal photosensitizer for photodynamic therapy in atherosclerosis is an unresolved question
- The role of indocyanine green as a photosensitizer in photodynamic therapy for atherosclerosis deserves further research.

Abstract

Aim

The aim of this article is to summarize and review the use of photodynamic therapy for the treatment of atherosclerotic plaque and the prevention of intimal hyperplasia. Different photosensitizers are discussed and more specifically the role of indocyanine green as a potential photosensitizer.

Methods

Literature search with focus on the use of photodynamic therapy in atherosclerosis, the mechanism of action and the different photosensitizers for photodynamic therapy.

Results

In-vitro and in-vivo studies confirm the possibilities of using photodynamic therapy for the treatment of atherosclerosis and the prevention of restenosis. Insufficient specificity in the accumulation of photosensitizer and thus phototoxicity, remains an important problem. Indocyanine green is a photosensitizer with features in favor of photodynamic therapy. Results obtained so far of photodynamic therapy with indocyanine green point towards the potential of indocyanine green as a photosensitizer in photodynamic therapy for atherosclerosis.

Conclusion

Photodynamic therapy is a promising tool for treating atherosclerosis. Many of the studied photosensitizers have toxic effects. Indocyanine green might be a good photosensitizer for the use of photodynamic therapy in atherosclerosis. These data justify further research to the use of indocyanine green as a photosensitizer in the treatment of atherosclerotic plaque both de novo or in restenotic lesions.

Keywords Atherosclerosis; Intimal hyperplasia; Photodynamic therapy; Photosensitizers; Indocyanine green.

Introduction

Atherosclerotic cardiovascular disease is a worldwide health problem associated with high morbidity, mortality and cost of care. The burden of disease remains high in both developed and developing countries due to the increased prevalence of atherosclerosis risk factors. Major risk factors include smoking, hyperlipidemia, hypertension, diabetes and aging. Lower extremity peripheral arterial disease affects approximately 200 million adults worldwide. The prevalence of peripheral arterial disease increases progressively with age. As a result, peripheral arterial disease is a growing clinical problem due to an aging population. Cigarette smoking correlates significantly with cardiovascular disease. On average, a diagnosis of peripheral arterial disease is made approximately a decade sooner in smokers than nonsmokers. Hypertension together with smoking is a major factor for progression of peripheral arterial disease in patients with diabetes mellitus. Diabetes patients have more advanced arterial disease at initial diagnosis and poorer outcomes than nondiabetic patients. Treatment of hyperlipidemia decreases the risk of progression of peripheral arterial disease [1-3]. Peripheral arterial disease results in impaired oxygen delivery to leg tissues. Most people with peripheral arterial disease will never experience critical limb ischemia. However, even without critical limb ischemia, patients with peripheral arterial disease have significant functional impairment that manifests itself as poor walking capacity and thus poor physical activity [4].

The desire to alleviate symptoms and to maintain function with minimal morbidity and reduced hospitalization in patients with peripheral arterial disease has led to an upward trend in endovascular lower extremity arterial procedures. Percutaneous transluminal angioplasty has a high initial success rate. Stents prevent early elastic recoil and late constrictive remodeling, and they are used to maintain lumen volume when compromised by a flow-limiting dissection or residual stenosis after treatment with atherectomy or

balloon angioplasty [5]. Mechanical and pharmacologic adjuncts to percutaneous transluminal angioplasty may reduce dissections and improve patency. For example, drug-coated balloons have reduced stent use in shorter lesions, but longer and more complex lesions often require stenting [6].

Without exception, all interventions designed to treat atherosclerotic occlusive disease are prone to restenosis. Restenosis severely limits the overall efficacy of these interventions and can occur in up to 80% of patients. The process involves a complex cascade of reactions that result in luminal narrowing through a combination of neointimal hyperplasia and constrictive remodeling [7-9].

The prevention and treatment of restenosis in the lower extremity are among the greatest unmet challenges in vascular surgery. Among several new therapeutic approaches to tackle the problem of restenosis, photodynamic therapy presents a promising alternative. Photodynamic therapy has the capacity to eradicate inflammatory cells, promote vascular healing and debulk the plaque. This therapy can be an interesting alternative to the use of balloons or stents.

Principle of photodynamic therapy

Photodynamic therapy is an emerging minimally invasive therapeutic technique for the treatment of a variety of diseases. It involves a combination of light, photosensitizer and oxygen dissolved in the tissue being treated. When photosensitizers are administered, they accumulate to a greater degree in hyperproliferating cells, such as those in malignant tumors and atherosclerotic lesions, than in normal cells The mechanism by which different photosensitizers localize selectively in the tissues targeted for therapy are not fully understood. Increases in vascular permeability coupled with an affinity for proliferating endothelium, are likely to contribute to their accumulation in the tissue [10,11]. The photosensitizer remains dormant until it is activated by light of a very specific wavelength [12]. The light that activates the photosensitizer is produced by a light source and is often transmitted through specially modified fiber optics. The resulting photochemical reaction mainly destroys diseased cells because the surrounding normal tissue, containing little or no photosensitizer, absorbs little light and is thus spared injury. Selectivity renders photodynamic therapy appealing in peripheral arterial disease, in which other endovascular approaches carry a substantial risk of damage to the normal arterial wall. Recent advances in laser technology and endovascular light delivery systems have broadened the scope of photodynamic therapy especially for atherosclerotic applications [13,14].

Mechanism of photodynamic therapy action

Photodynamic therapy demands three basic elements: 1) photosensitizer with selectivity for the target tissue, 2) a light source causing activation of the photosensitizer and 3) oxygen dissolved in the tissue being treated.

The photodynamic therapy response begins with accumulation of photosensitizer in the target tissue. The tissue is illuminated at the wavelength that favors light penetration into the tissue and subsequently causes activation of the photosensitizer. The photosensitizer serves as a catalyst for energy transfer. Light absorption leads to the release of cytotoxic singlet oxygen and release of other reactive oxygen species (Fig. 1). It is believed that this molecule is responsible for the subsequent vessel structural and functional alterations [15]. The resultant photobiological response is direct cell apoptosis and delayed necrosis from

neovascular damage. Apoptotic death can occur quite rapidly after photodamage, with cleavage of genomic DNA sometimes detected minutes after irradiation. The time interval between initiation of damage and vascular events vary from tissue to tissue and with different sensitizers. The time course of tissue response to photodynamic therapy can also be manipulated by varying the time interval between drug injection and light delivery and/or drug dose [16,17]. The vascular mechanism of photodynamic therapy is efficient in the case of cancer treatment. Microcirculation dysfunction is induced by vessel occlusion and vessel collapse, causing tumor shutdown. In addition to apoptosis and necrosis, autophagy is another death pathway associated with photodynamic therapy. Autophagy appears to be pertinent in cells unable to undergo apoptosis. Especially when lysosomes are the photodynamic therapy target, lethal photodamage is initiated because lysosomes participate in some steps of autophagy [18]. A particular photosensitizer can get localized in more than one organelle resulting in concurrent activation of more than one cell death pathways. Combining different photosensitizers in a photodynamic therapy protocol can also be interesting because different death pathways are activated. Cincotta et al [19] reported that they could eradicate large tumors in mouse by combining two different photosensitizers. One photosensitizer targeted lysosomes and mitochondria while the second had strong affinity for mitochondria and the endoplasmatic reticulum.

The main role of photodynamic therapy in the treatment of atherosclerotic plaque is to generate reactive oxygen species that interfere with cell survival and thus the remodeling process. An important feature of photodynamic therapy is that we only notice cell death in those illuminated areas in which there is adequate presence of the photosensitizer. Severe cellular damage is noted in atheromatous areas after light exposure. However, there is preservation of the intact elastic lamina and of normal collagen in the adventitia, suggesting that the integrity of the vessels is conserved. The extensive cell death is consistent with the regression of the atherosclerotic plaque [20,21]. In addition to plaque regression, photodynamic therapy can be applied to prevent restenosis. Restenosis is a complex mechanism that includes plaque collapse, vasoconstriction, thrombosis and remodeling, and proliferation of smooth muscle cells [22,23].

Following endothelial injury, there is vascular smooth muscle cell proliferation and migration resulting in intimal hyperplasia and narrowing of vessel lumen (Fig. 2). Reducing or inhibiting smooth muscle cell migration and proliferation is an effective treatment in the prevention of restenosis. Photodynamic therapy leads to inactivation of basic fibroblast growth factor thus inhibits smooth muscle cell proliferation and migration thereby preventing neointimal hyperplasia [24,25].

Cardiovascular Applications of photodynamic therapy

The role of photodynamic therapy is twofold. Photodynamic therapy has been tested for treatment of atherosclerotic plaque (Table1) but it can also be applied to prevent restenosis (Table 2) [14,26].

Photodynamic therapy for atherosclerosis

Selective accumulation within the atherosclerotic plaques was demonstrated with porphyrin based photosensitizers. In a rabbit atherosclerosis model, hematoporphyrin uptake was observed through the thickness of the plaque with a concentration gradient from luminal surface towards aortic wall [27]. In this study hematoporphyrin photodynamic therapy inhibits smooth muscle cell growth and decreases intima/media ratio of atheroma after 7 or 14 day post photodynamic therapy as compared with the control group. Major drawbacks for the use of hematoporphyrin photosensitizers in clinical setting are the cutaneous photosensitivity and inadequate penetration of 630-nm light through endoluminal blood [28,29]. Therefore, other photosensitizers were developed and tested.

Verteporfin, a benzoporphyrin derivative is a so-called second-generation photosensitizer[30]. It is a photosensitizer initially used to eliminate abnormal growth of leaky blood vessels in the eye caused by age-related macular degeneration and polypoidal choroidal vasculopathy, leading causes of vision loss in the elderly. Verteporfin was found to be taken up by atherosclerotic plaque of hyperlipidemic rabbits and miniswine. It is able to bind with endogenous low-density lipoproteins (LDL) and induces apoptosis when activated by light. Preassociation with LDL enhanced accumulation of this photosensitizer in atherosclerotic tissue when compared with normal artery. These studies show that selective uptake and retention of photosensitizer in atherosclerotic plaque is feasible and subsequent light activation of the accumulated photosensitizer could lead to highly selective removal of atherosclerotic plaque [31,32]. Phthalocyanines derivatives proved uptake by atherosclerotic plaques in rabbits. Rabbits with diet-induced atheromatous plaques were killed following intravenous administration of copper phthalocyanine tetrasulfonate. The concentration of the dye in atheromatous plaques was 2.6 times higher than in normal vessel wall [33].

5-Aminolevulinic acid is a precursor of protoporphyrin-IX. 5-aminolevulinic acid based photodynamic therapy for treatment of atherosclerotic plaque was conducted in rabbits and pigs. 5-Aminolevulinic acid-induced protoporphyrin-IX concentration was nine fold higher in plaque compared to normal aortic wall [34-36].

Motexafin lutetium is a member of the texaphyrin family, aromatic macrocycles that bear resemblance to porphyrins. Motaxafin lutetium shares the selectivity that allows porphyrins to accumulate in the atherosclerotic plaque but motaxafin lutetium absorbs light at longer wavelengths (>720nm). Photodynamic therapy with motexafin lutetium shows reduction in atheromatous plaque in both diet and balloon injury induced rabbit models of atherosclerosis [37].

Photodynamic therapy for intimal hyperplasia

In vitro investigations support the concept that photodynamic therapy inhibits growth of smooth muscle cells. Dartsch et al [38-39] demonstrated that smooth muscle cells from arteriosclerotic human arteries are destroyed by Photofrin in combination with ultraviolet light. Photodynamic therapy with chloroaluminum sulfonated phthalocyanine on bovine aortic endothelial cell preparations in vitro induces changes in the extracellular matrix: smooth muscle cell proliferation and migration are inhibited and endothelial cell proliferation is enhanced. These induced responses may be beneficial to vascular remodeling

and reduce the restenosis response [40]. LaMuraglia et al [41] even showed that there is preferential uptake of the photosensitizer phthalocyanine in the highly cellular regions of restenosis, thus suggesting that photodynamic therapy can be applied to both therapy and prophylaxis of restenosis.
In vivo studies were performed in different animal models like rats, rabbits, swine and monkey [42-49]. They all have promising results concerning the use of photodynamic therapy but show great variety in optimal dose of used photosensitizer, time interval and used light source.
Ortu et al [45] used phthalocyanine to inhibit intimal hyperplasia in a rat carotid injury artery model. Similar work was done by the group of Eton et al [46,47]. Photofrin was able to diminish intimal hyperplasia 1 week after endothelial injury. The same observation, 1 to 2 weeks after endothelial injury, was established by Asahara et al [48] in their rabbit model when using hematoporphyrin. A study from Hsiang et al [50] emphasizes the fact that photodynamic therapy after intimal injury should be given within the first week after endothelial injury, before significant intimal hyperplasia has occurred. Uptake of photosensitizer photofrin in balloon injured external iliac arteries of New Zealand White rabbits was minimal 2 weeks after injury.

Several clinical studies were conducted to show the safety and efficacy of photodynamic therapy in the prevention of restenosis. In 1999, Jenkins et al [51] performed the first clinical trial. To prevent restenosis after angioplasty, 7 patients underwent adjuvant arterial photodynamic therapy using oral 5-aminolevulinic acid. Mild side-effects as nausea and facial erythema have been described. The study suggested that photodynamic therapy is safe and no evidence of restenosis was observed during 6 months follow-up.
Rockson et al [52] investigated motexafin lutetium as photosensitizer in phase I and II trials. Patients received a single dose of motexafin lutetium before percutaneous intervention. The studies showed safety of motexafin lutetium photodynamic therapy. The need for selection of optimal drug and light dose is stressed. The optimal motexafin lutetium dose and light energy dose were defined in the study by Kereiakes et al [53]. Motexafin lutetium was administered to 79 patients by intravenous infusion 18 to 24 hours before percutaneous coronary intervention with stent deployment. Photoactivation was performed after balloon predilatation and before stent deployment. Motexafin dose 2 to 3mg/kg and light fluence 100 to 400 J/cm-fiber were safe and well tolerated. Most commonly observed side effects were peripheral paresthesia and rash, but these were generally mild and self-limited. No serious adverse events related to administration of the photosensitizer or endovascular illumination were observed. No adverse angiographic outcomes were observed at up to 6 months. This phase I drug and light dose-escalation study of phototherapy with motexafin lutetium defines important parameters for the application of photodynamic therapy.
Restenosis was also evaluated with photofrin photodynamic therapy. Photofrin was locally delivered 10min before coronary stent implantation. An over-the-wire device designed for localized delivery of solutions through openings located in the balloon segment was used (Dispatch® catheter) and tested in 5 patients. Follow-up was uneventful, with absence of major adverse effects [54]. In this study, a local delivery device was used to reduce the systemic side effects as result from intravenous injection with a photosensitizer. Exposure of

plaque to a high local concentration of photosensitizer can reduce dose and time of exposure and diminish systemic side effects [55].
Especially in vascular photodynamic therapy, together with local delivery of photosensitizer, the light delivery is another crucial aspect. The group of Mizeret et al [56] developed an endovascular light delivery system using an cylindrical diffuser. The devices are 1mm external diameter with homogeneous distribution of light intensity. Major advantages of such a system are (1) no risk of local heating, (2) possibility of making long fiber tips with nearly any desired profile, (3) the structure is rigid but can also be bent at quite tight angles and thus be used for interstitial or intravascular photodynamic therapy. For intravascular photodynamic therapy, these cylindrical light distributors are introduced in the vessel through a catheter. The light distributor can be centered in the vessel's lumen with an inflatable balloon. Balloon catheters provide the benefit to interrupt blood flow and remove blood from the illuminated surface. As it helps minimizing blood presence in the treated areas, the absorption of the excitation light used for photodynamic therapy is decreased [29]. The development of new "perfusion balloon- catheters" helps to resolve this problem. These are double balloon devices where the drug is entered between the two balloons so the drug is only in contact with the lesion. The catheter permits blood to flow through a separate channel in a double-balloon construct and effective local drug delivery is followed by local light delivery (Fig.3) [14]. Light emission distribution is specific to the diffuser manufacturer. In hollow organs this can result in over treatment of tissue distal to the placement of cylindrical diffusers [57]. The emission characteristics of light delivery fibers needs to be considered as part of the treatment planning.
However, the plaque itself is also a source of heterogeneity and the length of plaque can be very variable. These properties cannot be determined before every treatment and it is to be expected that further development of light delivery devices will include that light dosimetry can be adjusted according to type/length of plaque to be treated.
In a study from Jain et al [55], it is demonstrated that intra-arterial drug and light delivery for photodynamic therapy using liposomal Verteporfin is feasible. It is stated that illumination length can vary from 1 to 7cm and even longer light/drug applications are possible. They hypothesized that "intra-arterial photodynamic therapy" would be compatible with percutaneous coronary intervention procedures. Combining percutaneous coronary intervention with photodynamic therapy may reduce restenosis and permit preventive treatment of non-stented vulnerable plaque in order to reduce further coronary events.

In conclusion, studies demonstrated photodynamic therapy is efficacious in the prevention of restenosis. Together with major improvements in laser and fiber optic technology, which make local intravascular drug and light delivery possible and as such eliminates problems of insufficient drug/light selectivity, there is a promising role for photodynamic therapy in prevention of restenosis [58].

The choice of the photosensitizer

Effectiveness of photodynamic therapy depends on three components: oxygen, source of light with appropriate wavelength and photosensitizers.

Source of light and delivery of light are crucial in vascular photodynamic applications. Development of new light delivery systems make local illumination of plaque possible and support a regain of interest for vascular photodynamic therapy.

Appropriate choice of photosensitizer is one of the keys to success. For photodynamic therapy in atherosclerosis, de novo lesions and restenosis, the photosensitizer needs to target the plaque without harming the normal vessel wall. Accumulation of photosensitizer in the plaque is the most important factor associated with the photodynamic therapy effect. Other important features are 1) low or no toxicity without light 2) selectivity for the plaque macrophages 3) deep tissue penetration 4) targeted activation [15].

More than 400 compounds are known as photosensitizer. Depending on the type of disease and the affected tissue, the appropriate photosensitizers are used.

The first group, now designated as being the first generation of photosensitizers, are porphyrin macrocycles based on hematoporphyrin.

Porphyrin-based photosensitizers were the first of a number of photosensitizers with demonstrable, selective accumulation within the atherosclerotic plaque. There is maximal concentration in the intimal surface layers, diminishing radially into the media [59-60]. Despite selective damage of the plaque, hematoporphyrin and photofrin -a more purified derivative of hematoporphyrin- , there is lack of efficacy because 630nm light inadequately penetrates endoluminal blood [26,60]. Clinical application has also caused severe prolonged cutaneous phototoxicity. Prolonged cutaneous photosensitivity is the major late effect of concern. Acute erythema and edema, blanching and in severe cases complete necrosis of the skin is described. Skin photosensitivity can be diminished by rapid clearance of the photosensitizer due to excretion or sensitizer breakdown [12,16].

Newer second-generation agents with selective localization and minor cutaneous phototoxicity are available. Phototherapeutic capacity in atherosclerosis has been described for the phthalocyanines, chlorins, purpurins and benzoporphyrin (verteporfin) derivatives. Ideally such new derivatives would fulfill the photodynamic requirements in terms of improved physicochemical and therapeutic properties. But some of these photosensitizers, like phthalocyanines, still have major disadvantages like a slow clearance rate and poor aqueous solubility [16].To overcome these limitations, third generation photosensitizers are being developed. These third generation photosensitizers have the aim of improving the delivery of photosensitizer to the tissue and at same time enhancing specificity and efficiency of photodynamic therapy. Photosensitizers can be carried by vehicles including micelles, polymer nanoparticles, and self-assembled protein nanostructures, to form nanocomposites. Several reports have demonstrated that cellular uptake and bioavailability can be achieved by conjugating various ligands such as peptides or small compounds such as folate to the photosensitizer. The rationale is that certain tumor tissues show increased levels of specific cellular type receptor expression. These modified photosensitizer loaded nanoparticles are a promising new therapeutic strategy [60].

Several groups have been pursuing an optimization of photodynamic therapy applications by modification of the structure of the photosensitizer to obtain compounds with ideal hydrophobic/hydrophilic properties and increasing the selectivity of the photosensitizer by developing target photosensitizers [61].

With regard to noninvasive imaging of atherosclerosis, interesting work has been done by the group of Tsimikas et al [62]. They developed a novel approach that involves targeting oxidized low-density lipoprotein in the vessel wall. Vulnerable plaques that abruptly rupture, a major cause of sudden cardiac death, contain large pools of extracellular lipid, which is known to be rich in oxidized low-density lipoprotein. In experiments with hypercholesterolemic mice and rabbits, they showed that oxidation specific antibodies such as MDA2, a murine monoclonal antibody to malondialadehyde-lysine epitopes of oxidized low-density lipoprotein, has strong selectivity, specificity and high uptake within lipid-rich, oxidation-rich atherosclerotic lesions. The possibility of MDA2 antibody photosensitizer conjugates to selectively target atherosclerotic plaque can be interesting. The use of macrophage targeted photosensitizer conjugates is another example of a specific strategy to photoeradicate vulnerable plaques. Angiographically non-significant lesions that are excessively inflamed and characterized by large necrotic lipid cores, thin fibrous caps and dense macrophage infiltration are plaques vulnerable to rupture. The metalloproteinases released from monocyte-macrophages soften the plaque cap and promote them to rupture upon the cyclic mechanical stress of the luminal blood stream. The presence of macrophages has been advocated as a reason for preferential accumulation of photosensitizer in plaque. The use of macrophage targeted photosensitizer conjugates could give much higher selectivity for early plaque and vulnerable plaque that is particularly prone to rupture causing fatal cardiac events. It is however important to note that in atherosclerosis, a subset of macrophages, M2 macrophages, endothelial cells, and smooth muscle cells, are known to contribute in plaque stabilization. Their depletion, because of the phototoxic effect of photodynamic therapy, might provoke plaque rupture [63]. Improved selectivity for plaque macrophages is an advantage but an important point that needs to be considered is the lack of selectivity towards specific phenotype of plaque macrophages. Future photodynamic therapy should also focus on the distribution of the photosensitizer within the different compartments of the plaque. Development of photosensitizers still do not have the fully finished literature or research.

Studies regarding photodynamic therapy for atherosclerosis established photodynamic therapy as a treatment method for de novo or restenosis lesions. But the ideal photosensitizer for that photodynamic therapy is an unresolved question.

Indocyanine green as a possible photosensitizer for photodynamic therapy in atherosclerosis

Properties of indocyanine green

Indocyanine green (ICG) was developed in the Second World War as a dye in photography. In 1957 it was tested as a cyanine dye in human medicine. Initially it was used for determing cardiac output, and the hepatic function. ICG has been used for decades in ophthalmology for imaging retinal blood vessels. It is the only near-infrared dye approved by the USA Food and Drug administration for clinical applications. It is commercially available at low cost in a very pure form.

ICG is a tricarbocyanine, a negatively charged ion that belongs to the large family of cyanine dyes. (Fig. 4). ICG is a complex molecular structure and has amphiphilic properties, that is, both hydrophilic and lipophilic properties [64]. It is soluble in water (1mg/mL) but not readily soluble in saline. Therefore, ICG should be first dissolved in water and only after this diluted with saline if an isotonic solution is needed. But it is also well-known that in aqueous solutions, ICG molecules tend to aggregate. The absorption and fluorescence spectrum of ICG is in the near-infrared region. Depending on the solvent and the dye concentration, ICG absorbs mainly between 600nm and 900nm and emits fluorescence between 750nm and 950nm (Fig. 5 - Fig. 6). The peak optical absorption wavelength of ICG is approximately 800nm in blood plasma, with a fluorescence wavelength of approximately 810nm in water and 830nm in the blood. ICG works in the so-called “tissue optical window”. For tissue measurement, hemoglobin and water are the main absorbance molecules. Hemoglobin strongly absorbs light at wavelengths shorter than 650nm, while water absorbs light at wavelengths longer than 900nm. The wavelength between 650 and 900nm, termed the “optical window”, has a high transparency because of low light absorbance by hemoglobin and water. The peak excitation and emission wavelengths of ICG are 800nm and 830nm in blood, which are both within the optical window. Thus, the ICG fluorescence method can be used to provide assessment of blood vessels located relatively deep in the tissue [59,60]. Because ICG has amphiphilic characteristics, it binds to many plasmatic proteins and thus ICG is able to remain intravascular for a long time. Its fast binding to plasma proteins does not seem to alter protein structures, which is one sign of nontoxicity [65]. Since ICG is also not absorbed by the intestinal mucous membrane, it has very low toxicity. The safety of intravenously applied ICG in humans is very well documented with severe adverse reactions only in 0.05% of the cases [65]. The half-life is 2 to 4 minutes and it is removed from the circulation exclusively by the liver to bile juice. This short life time allows repeated applications. It must be stressed that ICG aqueous solutions are not very stable.
In aqueous solutions, ICG molecules tend to aggregate, which influences their optical properties. The relationship between absorbance and concentration of ICG is not linear, it does not follow Lambert-Beer’s law because of aggregation. The aggregation depends on concentration and time. The spectral stabilization is fastest when ICG is dissolved in distillated water, which can be useful when in certain examinations fast spectral stability is needed. In tissues and cells the NIR absorption peak, due to binding with cell proteins, is moved to longer wavelengths. Degradation is accelerated by exposure to light and high temperatures [66,67].

After light absorption, an excitated ICG molecule can follow three main pathways [68,69]. Firstly, the absorbed energy can be converted to a fluorescence emission. Maximum fluorescence is around 810nm in water and 830nm in blood. Fluorescence quantum yield of ICG in [solvant] is 4%. Discussion about the yield of fluorescence of a fluorophore involves complex concepts about scattering, reflection, transmission, absorption of the emission, and excitation light on the tissue molecules. Fluorescence yield of ICG increases linearly with the concentration in whole blood up to 80ug/ml. For concentrations above 80ug/ml, the fluorescence yield decreases. This is mainly explained by the aggregation process: ICG polymers have a weaker yield of fluorescence than ICG monomers [68].

Secondly, a part of the energy is transferred to an ICG triplet state by intersystem crossing. This triplet formation generates reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical. For ICG, it seems that these reactive oxygen species plays major role compared to other photosensitizers where singlet oxygen is predominantly produced [70]. The measured quantum yields of triplet formation by intersystem crossing was respectively 14%, 16% and 11% in water, methanol and aqueous albumin solution [64]. Engel et al [71] studied the stability of ICG when exposed to different light sources. Independently of the light source used, they suggest that ICG first produces singlet oxygen, although this was not detected, then further decomposition gives products that are responsible for the destruction of the cells. The mechanisms of actions are not clear because there is no direct proof of singlet oxygen.
The singlet oxygen quantum yield of ICG at different concentrations in different cell lines was studied in a publication from Ruhi et al [72]. The results show that higher concentration of ICG generates less singlet oxygen because of aggregation of ICG molecules. Aggregated molecules will still absorb the light but due to change in photochemical and photophysical properties of aggregated ICG molecules, there is decrease in singlet oxygen production.
Thirdly, the energy can be transformed into heat inside the ICG molecule. It is a therapeutic procedure that can be used to achieve a specific tumor killing effect This is the major process after photon absorption because about 85% of absorbed light energy is converted to heat. Aggregation of ICG may even increase the percentage of the absorbed light energy above 85% [68].

Because of its unstable optical properties due to degradation and aggregation, and its nonspecific localization, ICG can be encapsulated in nanoparticles. Nanoparticles are carriers for example for anti-cancer agent delivery but also protect the encapsulated drug against enzymes and hydrolysis. Yaseen et al [73] showed that encapsulation of ICG within dextran-coated mesocapsules protected ICG from thermal degradation at different tested temperatures. Due to their size, nanoparticles allow selective accumulation of the photosensitizer in the target cells. This could improve therapeutic applications of ICG because significant quantities at targeted sites can be achieved.

ICG applications

ICG is mainly used as a fluorescent probe [69]. Established medical applications of ICG are retinal angiography, liver clearance test, and cardiac output monitoring.
Recent interest in ICG is based on new applications in surgery, especially in angiography related to intraoperative monitoring of blood circulation in vital organs.
ICG angiography used intraoperative is simple and devices are cheap. ICG is given as a bolus injection into the blood circulation and imaging is done a few minutes after injection. For example in micro neurosurgical vascular operations ICG angiography is very useful. In the exclusion of intracranial vascular malformations ICG angiography is able to verify that the malformation has been completely removed but normal blood flow remains uncompromised [66].

ICG imaging in vascular surgery

In vascular surgery ICG is used as an imaging tool in 3 relative new domains.

ICG perfusion imaging in vascular surgery has been studied in a perfusion rat model to diagnose peripheral arterial occlusive disease. Perfusion rates of lower extremities with severe peripheral arterial occlusive disease was significantly lower than those of normal controls [74].
ICG fluorescence imaging in the assessment of critical limb ischemia is a recent method of imaging the foot perfusion. In a pilot study from Venermo et al [74], 41 patients with critical limb ischemia underwent ICG-fluorescence imaging after injecting ICG into the cephalic vein. ICG fluorescence imaging was quick to perform, easy to repeat due to rapid clearance of ICG and well tolerated. ICG fluorescence imaging in critical limb ischemia can be used to assess circulation at different sites, for example, at wound areas or the plantar side of the foot, which is not that easy to perform with transcutaneous oxygen pressure. Direct correlation of ICG fluorescence imaging with traditional measures, transcutaneous oxygen pressure or ankle brachial index, varied among the methods. This can be understood because all these methods measure circulation at different sites. However a strong correlation, especially in diabetic patients, was found between ICG fluorescence imaging and transcutaneous oxygen pressure. This can be explained because these two methods reflect local skin blood supply and media sclerosis does not have an impact on the results. ICG fluorescence imaging is therefore an additional tool in assessment of critical limb ischemia.

In lymphedema, ICG lymphangiography is popular as it clearly visualizes superficial lymph flow without radiation exposure. Even for evaluation of the effect of therapeutic compression in limb lymphedema, ICG lymphangiography is used [75].

Intravascular NIR fluorescence technology is used together with ICG to identify high-risk plaques. Lipid rich vulnerable plaques are the main cause of acute vessel occlusion in atherosclerosis. Methods are needed to detect coronary arterial plaques that are at risk for rupture. The group of Vinegoni et al [76,77] demonstrated that ICG could rapidly target lipid-rich atheroma in cholesterol-fed New Zealand white rabbits.

In summary, these studies demonstrate that ICG imaging is a very powerful tool. Use of ICG to evaluate perfusion and to identify vulnerable plaques is promising but additional ICG dosing and timing studies are further needed. Once these results are obtained, it is expected that clinical application of ICG imaging in vascular surgery will increase.

ICG for photodynamic applications
ICG is mainly used as a fluorescent dye but has also been proposed as a photosensitizer. In combination with light it causes both in vitro and in vivo cytotoxic effects.
Publications with the use of ICG for photodynamic applications are numerous. In very common skin diseases as acne vulgaris, ICG and a diode laser has proven to be a very effective therapy [68]. Multidrug resistant bacteria as Pseudomonas aeruginosa are killed with photodynamic therapy and ICG as a near infrared photosensitizer. Recent publications describe the use of ICG for photodynamic therapy in multidrug resistant cancer and in treatment of nosocomial respiratory infections caused by resistant pathogens [78-80]. These

are examples of what is called deep-tissue photodynamic therapy, photodynamic therapy with a photosensitizer active in the near infrared. These near-infrared wavelengths are able to penetrate deeper into human tissue compared with the penetration of visible light [65]. The photodynamic effect of ICG is however still relative unclear. In a publication from Giraudeau et al [68] it is mentioned that there is cell destruction due to the production of reactive oxygen species but also an important thermal effect which causes selective photocoagulation. Moreover, comparison between the conducted studies with ICG as a photosensitizer is difficult because of the great variety in the parameters like different ICG concentrations.

Although difficult to compare, it is clear that ICG for photodynamic applications is effective and well-tolerated. This is mainly due to basic properties like its amphiphilic characteristics and specific pharmokinetics (Table 3). Limitations of ICG for clinical applications like poor aqueous stability and lack of target specificity are solved by targeted photodynamic therapy. Several encapsulations have been implemented with ICG. Makino et al [81] have labeled lactosome with indocyanine green. The labeled lactosome was found to be stable in the blood circulation and accumulated specifically at a mouse model liver tumor site.

There are few data regarding the use of ICG as a photosensitizer in atherosclerosis. A preliminary study to understand the effect of ICG photodynamic therapy in balloon-injured carotid arteries of 15 rats was conducted by the group of Lin et al [82] .

A balloon injury model was used to induce intimal hyperplasia of carotid artery. Photodynamic therapy was performed 7 days after balloon injury and in a small group, this therapy was again repeated after 7 days. ICG was injected into the tail vein 1 hour before light irradiation. External illumination with 780-nm light-emitting diode light was applied. Results showed that two photodynamic treatments effectively decrease arterial wall thickness, and intimal hyperplasia area, as well as prevent the reduction of lumen diameter after angioplasty. This study supports the photodynamic effect of ICG and the rationality of the photodynamic effect relating to the number of therapy sessions.

In a more recent study conducted by the same group, the effect of photodynamic therapy with ICG and near infra-red irradiation on the viability of vascular smooth muscle cells was studied [83]. Smooth muscle cells play an important role in the pathogenesis of restenosis. It was demonstrated that photodynamic therapy using ICG and near infra-red light illumination is effective in suppressing viability and enhancing death of smooth muscle cells. They concluded that further studies are needed to clarify the pathway responsible for apoptosis resulting from ICG photodynamic therapy.

Conclusion

In spite of the convincing evidence of in-vitro and in-vivo studies which confirmed the ability of photodynamic therapy to treat de novo or restenotic atherosclerotic lesions, this treatment has remained a research tool. Several parameters still require further research such as optimal concentration of photosensitizer, light source and tissue oxygen condition. The ideal photosensitizer for the use of photodynamic therapy in de novo or restenotic atherosclerotic lesions is still an unresolved question. Many of the studied photosensitizers

have toxic effects of which a severe skin photosensitivity reaction is one of the most important.
ICG is a very powerful dye with long history and use in different clinical applications, mainly as a fluorescent probe. One of the recent studied fields is the imaging of lipid-rich plaques by near infrared fluorescence with the use of ICG. In photodynamic therapy, use of ICG as a safe photosensitizer is well documented.
Combining its properties as a very good imaging tool in atherosclerotic plaque and its capacities as photosensitizer in photodynamic therapy, ICG can be a good photosensitizer for the use of photodynamic therapy in atherosclerosis. Data available regarding the use of ICG as a photosensitizer in photodynamic therapy for atherosclerosis are promising but this definitely deserves further research.

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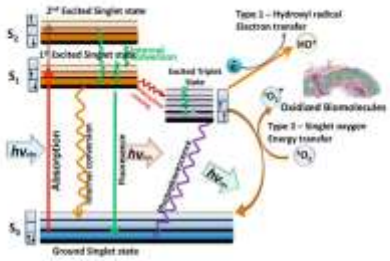
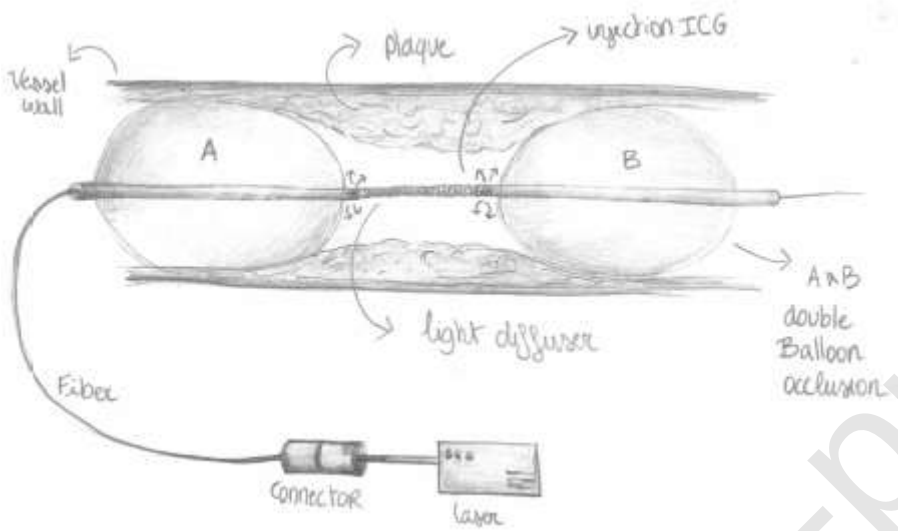
Legends for Figures :



- Fig. 1 Mechanism of action of photodynamic therapy including the Jablonski diagram.
When light (*hv*) is absorbed by the PS, the electron moves from a non-excited low-energy singlet state into a high-energy singlet state. This excited state can lose energy by emitting a photon (fluorescence) or by internal conversion (non-radiative decay). The process known as intersystem crossing involves flipping of the spin of the high-energy electron, leading to a long-lived excited triplet state. In the presence of molecular oxygen, superoxide and hydroxyl radicals are formed in Type I reactions and singlet oxygen in a Type II reaction. These ROS can damage most types of biomolecules. S0, ground state of the photosensitizer(PS); S1, first excited singlet state of PS; S2, second excited singlet state of PS; IC, internal conversion; 3O2, triplet oxygen; 1O2, singlet oxygen.
Reprinted from " Animal models for photodynamic therapy" by Zenildo Santos Silva, Jr, Sandra Kalil Bussadori, Bioscience Reports 2015 Dec; 35(6.

- Fig. 2 Balloon angioplasty induces production of Reactive Oxygen Species. Reactive oxygen species are generated by different enzymatic systems, namely nicotinamide adenine dinucleotide phosphate. Reactive oxygen species stimulate smooth muscle cells and fibroblast proliferation and migration through the internal elastic lamina to form a neointimal layer leading to restenosis.
Reprinted from "Oxidative stress and pathological changes after coronary artery interventions "by Rio P. Juni, Henricus J. Duckers. JACC 2013 April, vol 61.



- Fig. 3. Double balloon device. Double balloon device where the drug is entered between the two balloons. The catheter permits blood flow through a separate channel in a double balloon construct. Effective local drug delvery is followed by local light delivery.
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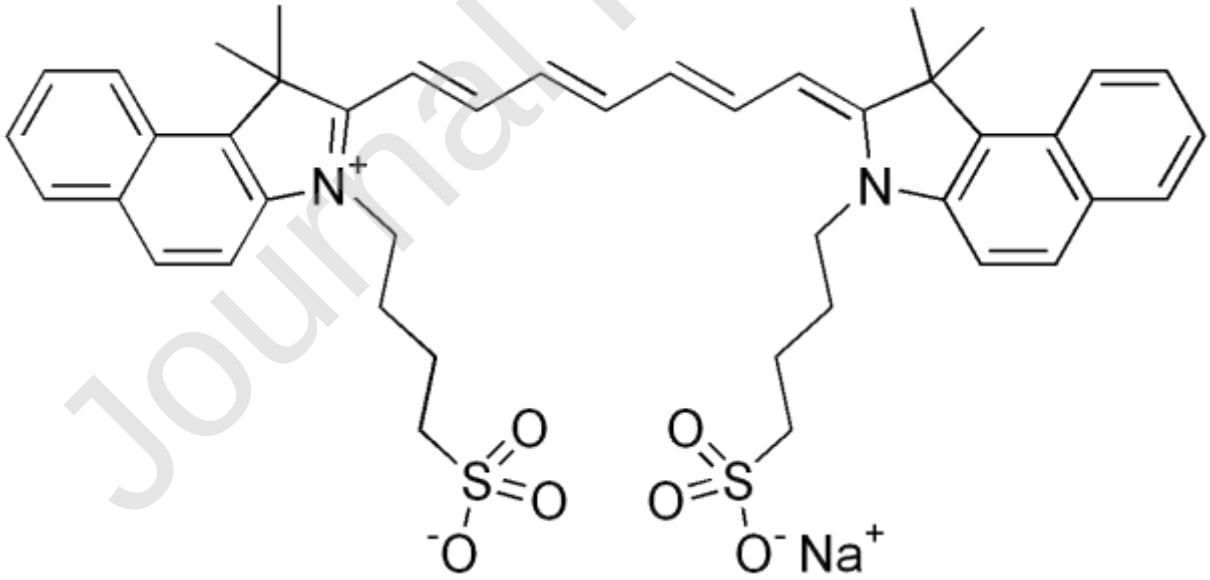
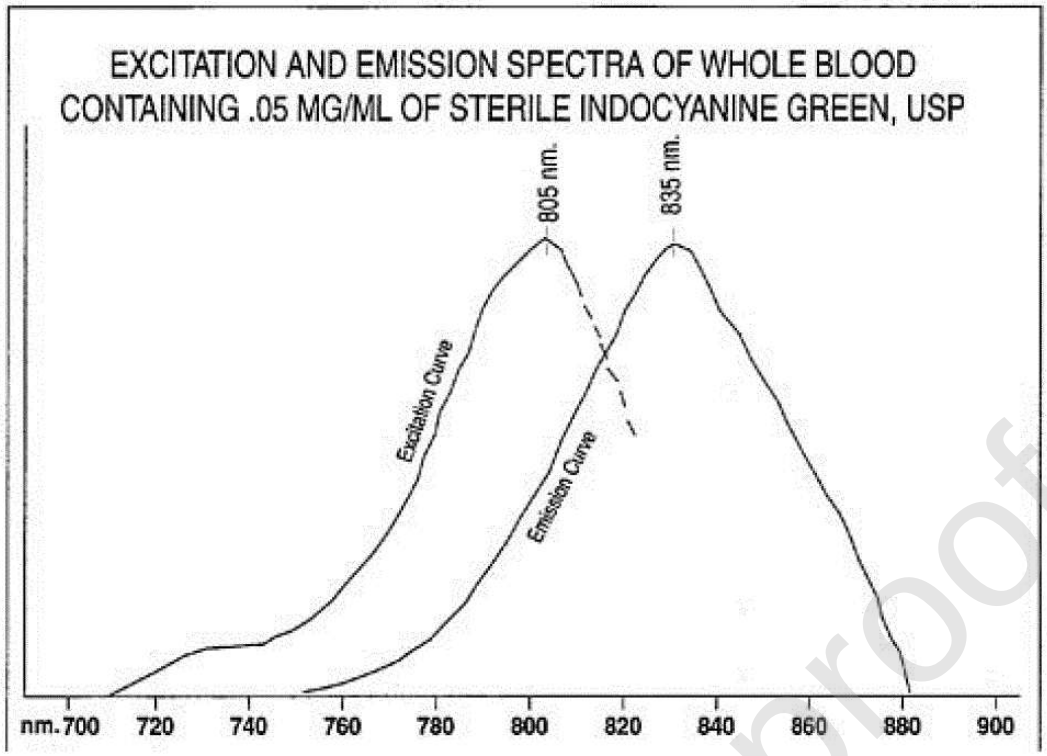


- Fig. 4 Chemical structure of Indocyanine green. Retrieved from Wikipedia, The Free Encyclopedia.
https://en.wikipedia.org/w/index.php?title=Indocyanine_green&oldid=835788250



- Fig. 5 Excitation and emission spectra of ICG in blood plasma

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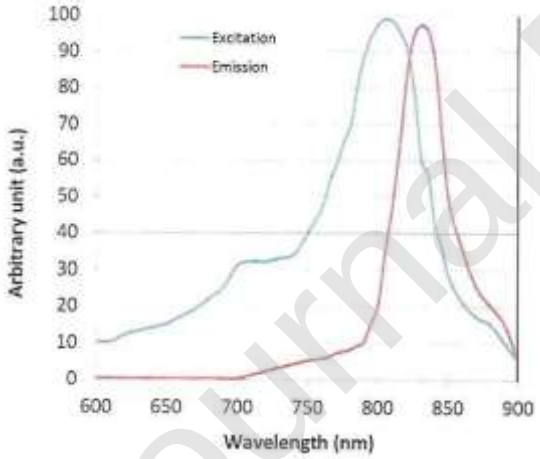

- Fig. 6 Excitation and emission spectra of ICG.

Depending on the solvent and the dye concentration,the maximal absorption is observed between 600 and 900nm. In blood plasma the maximal absorption is at approximately 800nm.

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Table 1 Studies demonstrating the selective uptake of photosensitizer in atheromatous plaque.

Photosensitizer	**Absorption spectrum**	**Animal model**	**Dose**	**Major findings**	**References**
Hemato-porphyrin,photofrin	630nm	Rabbits Monkey	2.5mg/kg IV	Accumulation in plaque	[27]
5-aminolevulinic acid	630nm	Rabbits Pig	60-120mg/kg IV	Uptake in plaque Reduces atherosclerosis progression	[34,35,36]
Phthalocyanine derivatives	670nm	Rabbits	2.5-5mg/kg IV	Uptake in plaque	[45]
Verteporfin	692nm	Rabbits Miniswine	2mg/kg IV	Uptake in plaque	[30,31]
Motexafin lutetium	710nm	Rabbits	1.2mg/kg IV or local perfusion	Uptake in plaque Decrease in atheroma burden	[13]

Table 2 Studies demonstrating the effect of photodynamic therapy in management of restenosis.

Photosensitizer	**Absorption spectrum**	**Animal model**	**Dose**	**Plaque localization : Plaque: normal artery ratio**	**Major findings**	**References**
Hemato-porphyrin, photofrin	630nm	Rabbits Miniswine Stented human artery	2.5-5mg/kg IV	Plaque: normal artery ratio of 3:1	Photodynamic therapy prevented intimal hyperplasia No adverse effects No in-stent restenose humans	35,37,40,44,46, 50
5-aminolevulinic acid	630nm	Rabbits Pig	60-120mg/kg IV	Plaque: normal artery ratio 9 to 12:1	Photodynamic therapy prevented stent induced intimal hyperplasia	34,35
Phthalocyanine derivatives	670nm	Rabbits Rats	5mg/kg IV		Photodynamic therapy prevented neointimal hyperplasia	41,45
Verteporfin	692nm	Rats Miniswine	2mg/kg IV	Plaque :normal artery ratio of 1.7 to 3.5	Photodynamic therapy prevented neointimal hyperplasia	40

Table 3 Possible advantages and disadvantages of ICG as photosensitizer.

Advantages of ICG
- Absorption and Emission in near infrared → deep tissue penetration - Only near infrared dye approved by Food and drug administration - Low or no toxicity - Binds to many proteins →stays intravascular for long time - Short half- life → repeated applications possible - Cost-effective - Atheroma-targeting capability - ICG encapsulations → more specific photodynamic therapy - Long use in different clinical applications →facilitates introduction to new applications
Disadvantages of ICG
- Lack of selectivity - Low quantum yield of reactive oxygen species - Rapid degeneration in moist environments - Instability in function of concentration, pH, temperature and light exposure - Fast binding to albumin and high-density lipoproteins, causing agglomeration