A Fast Test System for the Effect of in-vitro Parameters on the Sensitizing Efficiency in Photodynamic Laser (Tumor) Therapy¹

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Key Words

Photodynamic therapy

Photosan III

Singlet Oxygen

In-vitro test system

Summary

An optimized application of the photodynamic laser therapy for cancer treatment requires the knowledge of the effect of in-vitro parameters, such as biochemical targets and irradiation protocols, on the sensitizing efficiency. Sensitizers such as hematoporphyrin-derivatives react mainly via singlet oxygen formation with proteins and lipids within the cells when activated by light having the appropriate wave length. Singlet oxygen and oxygen radical formation can be determined by oxygen consumption of the reactants. An existing oxygen consumption measurement system was adapted for fast determination of the reactivity of the different parameters. Two examples of the applicability of the fast screening test system are presented here: Variation of the sensitizer to target ratio and the light intensity.

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Schlüsselwörter

Photodynamische Therapie

Photosan III

Singulett Sauerstoff

In-vitro Test-System

Zusammenfassung

Eine optimierte Anwendung der Photodynamischen Lasertherapie für Krebsbehandlung erfordert Kenntnis von der Wirkung der in-vitro-Parameter wie biochemische Targets und Bestrahlungsprotokolle auf die Sensibilisierungs-Effizienz. Sensibilisatoren wie Hämatoporphyrinderivate reagieren hauptsächlich über Singulett-Sauerstoff-Bildung mit Proteinen und Lipiden innerhalb der Zellen, wenn sie durch Licht einer geeigneten Wellenlänge aktiviert werden. Da Singulett-Sauerstoff und Sauerstoff-Radikal-Bildung am Sauerstoffverbrauch der Reaktanten gemessen werden kann, wurde ein bestehendes Meß-system für Sauerstoffverbrauchsmessungen für die rasche Bestimmung der Reaktivität der verschiedenen Parameter adaptiert. Zwei Beispiele für die Anwendbarkeit des Schnelltest-Systems werden hier vorgestellt: Die Veränderung des Sensibilisator-Target-Verhältnisses und der Lichtintensität.

Introduction

The efficiency of the photodynamic laser therapy (PDT) is dependent on the selective uptake of photosensitizers by tumor tissue, followed by irradiation with light of a sensitizer-activating wave length. This results in the generation of cyto-toxic products, capable of destroying tumor cells. When administered to patients by intravenous injection in clinical therapy, the sensitizer

accumulates at a higher rate in tumor tissue than in normal tissue and/or is cleared slower from tumor tissue. The mechanism of selectivity is strongly suggested to be due to the changed vascularisation and metabolism of tumor tissue. At present, hematoporphyrin derivatives (HPD) and those active fractions with trade names such as Photofrin® or Photosan®, are officially admitted for clinical application. Although those products display an absorption maximum in the visible blue spectrum (about 400 nm), a small peak in the visible red range (about 630 nm) is used for excitation by irradiation, since red light penetrates deeper into tissue than light with shorter wave lengths. Tumor irradiation is usually carried out 24 to 72 h after drug administration by low-energy dye lasers; they are equipped with fiber optics and applicators for the different tumor sites, especially in hollow organs [1, 2]. In preclinical studies, artificial sensitizers, such as phthalocyanines and chlorins, are currently being investi-

This study was supported by: Akademischer Senat der Universität Salzburg
gated. They exhibit some advantages, such as an absorption maximum in the red spectrum. The photodynamical process requires, besides sensitizer and light source, suitable biochemical targets and the presence of molecular oxygen. While a blue light activated sensitizer, such as HPD, decays to the ground stage by emission of red fluorescent light, which is helpful in diagnosis, excitation by red light can be used for the therapy due to the following molecular mechanism: Activated HPD molecules change from an excited singlet state to the reactive triplet state by inter-system crossing. Next, they transfer their energy (without emission of radiation) either to different substrates, which generate free radicals (type I reaction) or to molecular oxygen, which is activated to singlet oxygen (type II reaction). All aggressive products, free radicals and singlet oxygen, induce cytotoxic reactions with proteins, lipids, nucleic acids, etc., within the different cellular targets. HPD molecules act mainly via type II reaction [1, 2]. As a measure of the effect of the reactants on the sensitizing efficiency, the formation of reactive and toxic intermediate products (type I and II pathway) may be taken. Because this process requires molecular oxygen, consumption of such within a closed system can also be used to determine sensitizing efficiency. The aim of the present study was to adapt a set-up for oxygen-consumption measurements as a test system for fast, reproducible measurements to record changes of these parameters: Sensitizer, target, light, wave length and oxygen [3].

Fast in-vitro Test System for Photodynamic Action

463

%/min

Material and Methods

Photosensitizer: Photosan III (PSIII; Seehof-Laboratories, Wessel-burener Koog, FRG), the active compounds (di- and oligomers) of HPD, is officially admitted for clinical use in Germany and Austria. Light source: Halogen lamp, which can be established with filters of different wave lengths. Target: Bovine serum albumin (BSA; 1.5 mM) as protein; albumin is a main component of the blood serum. The oxygen-consumption measurements were carried out by membrane-coated polarographic Clark-type electrodes (Pt) with integrated reference electrode (Ag/AgCl), in closed but light transmittable chambers of 0.6 ml volume. The measuring signal was amplified by a microsensor and expressed in percent of oxygen [4]. After calibration of the electrode in a solution with 0% and 21% oxygen, the chamber was closed and filled with the sensitizer-target-mixture. The measurement started in darkness. As soon as a constant value was reached, the light source was turned on, followed by the immediate onset of oxygen consumption.

Results

Several investigations of the sensitizing efficiency were carried out, using different sensitizers with different targets, different activating wavelengths and changes in environmental parameters. Two examples for changes of the oxygen-consumption rate due to changes in the sensitizer-target-ratio and in the light intensity are demonstrated as follows.

PSIII (concentration: 50 µg/ml) and BSA (1.5 mM) in different ratios was measured in darkness. No significant oxygen consumption could be observed. Immediately after the onset of white light exposure with 90 W/m², molecular oxygen was consumed until reaching a value close to 0%. Over a wide range of PSIII-BSA ratios, up to 1 to 9 for both variables, the reaction is almost independent of the concentration of both reaction partners. Portions smaller than 10% for either reactants induced increasingly slower oxygen consumption, with pure PSIII or pure BSA showing minimum values under irradiation. The 1 to 1 mixture of PSIII-BSA corresponds to physiological ratios within the body during PDT, where BSA is substituted by serum and PSIII is administered in concentrations of 2.5 mg/kg body weight (fig. 1).

PSIII (concentration: 50 µg/ml) and BSA (1.5 mM) are used in a 1 to 1 ratio. After recording a stable value with minimum oxygen consumption in darkness, the mixture was exposed to white light with different intensities. This resulted in a linear correlation between oxygen consumption rate and light intensity, exhibiting a linear part in the range of 66–154 W/m² (fig. 2).

Discussion

It can generally be concluded that the sensitizing efficiency of PDT is influenced by the presence of a sufficient amount of specific sensitizer and target molecules, and by oxygen and light with the activating wave length for the formation of reactive intermediate products [3].

Reactions of Photosan III (50 µg/ml) with BAS (1.5 mM) as target induce type I and II energy transfer with the formation of singlet oxygen and free radicals, and unspecific protein oxydation, thereby consuming molecular oxygen [3]. The ratio of both reactants does not significantly influence the consumption rate.

Fig. 1. Oxygen consumption rate (%/min) for different ratios of Photosan III and bovine serum albumin.

light intensity in W/m²

Fig. 2. Oxygen consumption rate (%/min) for irradiation of Photosan III (50µg/ml) and bovine serum albumin in a 1:1 ratio with different light intensities.

tion rate over a wide range, which indicates saturation of both sensitizer and target. In clinical PDT, the partial inactivation of the sensitizer in the blood system by serum in darkness before reaching the tumor sites should be considered. The linear dependence of the reaction of PSIII and BSA (1 to 1) on the intensity of the light source within the range of 66–154 W/m² affirms the importance of a precise dosimetry and a sufficient and uniform irradiation of the tumor by laser light.

Through these two examples we were able to demonstrate that the test system presented in this paper is suited for reproduction and fast screening measurements.

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