LASER APPLICATIONS IN BIO-MEDICINE:
TUMOR THERAPY AND LOCALIZATION USING PHOTOSENSITIZING DRUGS

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INTRODUCTION

Among the various applications of lasers in Biomedicine, a particularly interesting one makes use of a laser beam and photosensitizing drugs, for therapeutic and diagnostic purposes. Out of the several possibilities offered by this combination of laser light and drugs, we shall consider in this paper only the case of tumor therapy and tumor localization. The basic principles of these applications rely on the fact that a wide class of drugs present a high accumulation or retention in a tumor, or in a highly proliferative tissue, as compared with the surrounding normal tissue. For therapy the drug must also be able to induce a cytocidal reaction when activated by (laser) light of suitable wavelength. On the other side, for localization, when bound to the tissue, the drug must exhibit a reasonably high fluorescence quantum yield so that its emission can be detected by a suitable system. Thus it may happen that a drug suitable for tumor therapy is not suitable for tumor localization. In both these cases, however, two further conditions must be fulfilled:

(i) - the drug must be nontoxic (in the dark) and non-mutagenic;
(ii) - the drug must absorb and emit at wavelengths that can permit a reasonably good penetration into the tissues.

PHOTODYNAMIC THERAPY

With almost all the drugs this therapy is based on the photodynamic action, a photochemical reaction well known since the beginning of this century. In this "action" a suitable drug, henceforth called "photosensitizer", is first raised by a photon of appropriate energy to its excited singlet state \( S_1 \). A fraction of the excited molecules then undergoes intersystem crossing thus decaying to the triplet state \( T_1 \). Once in the triplet state the drug cannot decay radiatively to the ground state, since the triplet to singlet transition is forbidden by the selection rules. The
decay of the triplet state can therefore occur only by non-radiative decay. For a photosensitizer, this decay predominantly occurs by collision with oxygen, which is normally present in the tissue and which, in the ground state, is in a triplet level. After the collision, an energy exchange process may occur wherein the photosensitizer returns to its ground state and the oxygen is raised to one of its excited states. Since the total spin of the two interacting molecules must be conserved upon collision (Wigner's selection rule), the oxygen needs to be excited to its excited singlet state. Once in this state, the oxygen cannot decay radiatively to its ground state (singlet-to-triplet transition is forbidden) and, furthermore, it has sufficient energy to overcome the potential barrier for most chemical processes. This means that singlet oxygen can react with many cellular components, mostly at the membrane sites of a cell, to create photodynamic products which eventually lead to the cell death. The "photodynamic action" is thus the principle upon which the kind of tumor therapy described here is based: this therapy is, accordingly, called photodynamic therapy. Note that the energy transfer can occur provided that the triplet energy of the sensitizer is larger than or about equal to the energy of the excited singlet state of the oxygen. Since the corresponding wavelength of the latter energy is about $\lambda = 800 \text{ nm}$, this requires that the wavelength of the singlet to triplet transition ($S_0 \rightarrow T_1$) and hence that of the singlet to singlet transition, $S_0 \rightarrow S_1$, of the sensitizer be larger than this wavelength.

The most common drugs, currently used for the photodynamic therapy of tumors, are Hematoporphyrin Derivative (HpD) and the so-called DHE (Di-Hematoporphyrin Ether or Ester). They are commercially available under the trade names of Photofrin I and Photofrin II, respectively. These drugs are derived from Hematoporphyrin (Hp), a well known dye belonging to the wide class of porphyrins. Its chemical structure consists of four phenol rings which form the chromophoric group. HpD is formed from Hp through a standard chemical procedure which consists in acidification with sulfuric and acetic acid, followed by neutralization. DHE is prepared from HpD by a suitable procedure using gel filtration, thus obtaining a higher percentage of aggregate fractions. The absorption spectra of DHE and HpD in buffer solution, besides a strong absorption peak at about $\lambda = 365 \text{ nm}$ (the Soret band), present weaker absorption bands extended to the red (the last peak occurs at $\lambda \approx 615 \text{ nm}$ in a buffer solution and at $\lambda \approx 630 \text{ nm}$ when the drugs are bound to the cell components).

HpD and DHE are complex and still not completely unravelled mixtures of many compounds. This can be evidenced by high-pressure liquid chromatography. Chromatograms, where the optical density of the solution at $\lambda = 400 \text{ nm}$ (approximately correspond to the peak of the Soret band) at a given position in the distillation column is plotted vs distillation time, consist of three well defined peaks occurring in the early stage of the distillation, and a broad continuum occurring later. The peaks have been identified as due to Hematoporphyrin, (Hp), Hydrossiethylvinil deuteroporphyrin, (HVD), and Protoporphyrin, (PP), i.e. dehydrated Hp in monomeric form. The broad continuum likely consists of aggregates of the previous monomers, in dimeric or oligomeric forms. The monomers and the various aggregates occupy different positions along the distillation column and can thus be physical-