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Adventures in photodynamic therapy: 1976-2008

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Abstract

While the concept of photodynamic therapy dates from 1900, and there have been periodic re-discoveries, the clinical era really began with the studies by Dougherty and associates in the early 1970s. This report relates my encounter with the field of PDT, along with experimental approaches to the elucidation of pertinent phototoxic mechanisms.

Keywords

photodynamic; apoptosis; autophagy; reactive oxygen species

Introduction

Photodynamic therapy involves the use of light to bring about events that lead to the death of assorted cell types. The major ingredients consist of oxygen, a photosensitizing agent, a target cell or tissue and sufficient financial support. Use of light for the bleaching of linen has been traced to the early Egyptians, and the destruction of photosensitized micro-organisms was first reported in 1900 by Oskar Raab, a medical student venturing into the unknown. The current clinical era began with studies by Thomas Dougherty and associates at the Roswell Park Cancer Center in the 1970s.

Dougherty has written several descriptions of this era, along with updates, with perhaps the best being a chapter he has prepared for the book, 'Photodynamic therapy of diseases of the head and neck', edited by Merrill Biel, to be published in 2008 by Plural Press. My own introduction to the field occurred in 1976 when I received a letter from the National Cancer Institute, asking me to confirm an observation by Dougherty's group that a red-brown power could eradicate murine tumors. This was to be done under the terms of a contract I had with the NCI to examine any new drugs that were sufficiently unusual to warrant a closer look.

As it turned out, my prior background had prepared me for this effort. My original training was at MIT, where I learned about spectroscopy, fluorescence, fundamentals of chemistry, and similar topics that would be useful later (although I likely did not realize it at the time). A few students persuaded Prof. Bernard Gould to teach a course in biochemistry, which was probably the first in the history of MIT. This was a bizarre experience, since not much was known about this subject and there was a suspicion that protein synthesis involved enzymes like trypsin and pepsin running 'backwards'. All chemistry students were obliged to carry out a mini-thesis project and mine was done under the direction of John Sheehan, notable both for his role in the synthesis of the explosive RDX, and the drug penicillin. Sheehan was hesitant to recommend, for me, a career in organic chemistry, but suggested that I do graduate work somewhere else, perhaps where he had been trained: the University of Michigan. The organic

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“star” there, at the time, was R. C. Elderfield who was also producing a long series of volumes on the topic of Heterocyclic Compounds.

Intermediary Steps

Elderfield took me on as a prospective graduate student, and quickly deduced that I could follow directions, deal with lab. hazards and appreciate good ideas when I heard them. When it came to producing these good ideas, the data were not so clear. I did get a good background in organic techniques, but then fell under the influence of Cyrus Levinthal who was in Ann Arbor briefly en route to a remarkable career in microbial genetics at Columbia University. A glance at his record will readily confirm this, *e.g.*

<http://www.columbia.edu/cu/biology/faculty/chasin/cyrus.html>. His advice to me was to remember what I had learned about Chemistry, but to look into the rapidly advancing field of Biochemistry. My first thought was to investigate Biophysics, but he recalled the adage that the biophysicist talks biochemistry to physicists and physics to biochemists without a thorough understanding of either subject; this may no longer be true.

Based on his advice, I moved to the department of Biological Chemistry, but at that time, the only entry course was in the school of medicine. I must admit that this was unquestionably the worst organized and most poorly-taught course I had ever encountered, accounting no doubt for the low regard in which ‘biochemistry’ was held by medical students. Fortunately, this situation has been corrected, at least to some extent, by now, as it is realized how important biochemical principles are to the practice of medicine. At that time (1954-58), the biochemistry students at the University of Michigan represented a rare collection of individuals who would make major contributions to the field. These included Marshall Nirenberg, Nobel Prize winner for his deciphering of the genetic code, Edward Kravitz, now with an endowed chair at Harvard medical School, and Milton & Sondra Schlesinger who had highly productive careers at Washington University.

I next had a post-doctoral fellowship at Harvard, a place where almost everyone seems obliged to pass through at some point. At Harvard, and later at the Children's Cancer Research Foundation (CCRF), I learned about drug development, mammalian cell culture, fluorescence techniques and other items that would prove helpful in those PDT studies to come. At the CCRF, I was advised by the director, Sidney Farber, to go do something significant and he would then invite me back with a more permanent position. My group moved to the University of Rochester in 1967, a place that has become prominent in PDT work over the years, after I left. The Rochester experience was mainly concerned with more drug development and ended in 1974 when I was offered a position at Wayne State University as Professor of Pharmacology and Medicine, without ever having taken a formal course in either subject. The invitation to examine the mysterious photosensitizing agent from Roswell Park came two years later.

Photodynamic Therapy: Early Days

My group began reporting on PDT beginning in 1977 [1], and my first NIH award on the topic was received in 1983. Somehow, this has been renewed through 2010. Of the 74 current NIH grants with the term ‘photodynamic’ somewhere in the abstract, this appears to be the longest surviving example; in second place is C. J. Gomer with an award in its 24th year. As PDT began to develop, mainly based on the clinical data coming from Roswell Park, many conferences were arranged to spread the word. Dougherty organized a meeting in Buffalo in 1977 to bring together the world-wide group of clinical experimenters in the field and the NIH organized a conference in Bethesda a year later, mainly, I suspect, to find out if this was ‘for real’: the idea that a drug + light could eradicate cancer. The NIH also supported a meeting I organized in Washington in 1981, providing \$16,000 in support. In terms of 2007 dollars, this amount is quite impressive, amounting to approximately \$40,000. The average NIH support

for conferences is approx. \$5000 today. The NIH also supported another such conference in 1984. Reports from both have been published [2,3]. I organized several more conferences over the years, often in conjunction with the American Society for Photobiology. PDT is also a common topic at ASP and European Society for Photobiology meetings, as well as ICPP, Gordon Conferences and similar venues.

I had been unsuccessfully attempting to get Ciba (now Novartis) to hold a PDT symposium in London in the 1980s, but they did agree to a Discussion Group talk in 1982. This was attended by many of the leading porphyrin and photobiology experts in the UK. Among these was Sir Alistair Currie, author of the first report on apoptosis. He pointed out this reference and asked me whether PDT might not be acting as an apoptosis inducer. Since the primary means for identifying apoptosis at the time was the electron microscope, I had to defer the proposal since the NIH was not going to buy an electron microscope for me, and the users at Wayne State University had no interest in this idea. In 1991, Nancy Oleinick (at Case Western Reserve University) was able to establish that apoptosis was indeed a pathway to PDT photokilling. Ciba finally did sponsor a Foundation Symposium on PDT in 1989 [4].

Mechanisms and Processes

One of the early studies from my laboratory involved an examination of the nature of hematoporphyrin derivative (HPD). This was the first of the PDT drugs, initially prepared by Sam Schwartz. Schwartz was initially examining the ability of different porphyrins to act as radiation sensitizers, and later turned to the examination of hematoporphyrin samples of various degrees of purity to localize in neoplastic tissues. He eventually began working with Richard Lipson, a thoracic surgeon. Lipson later reported treating a patient with cancer using hematoporphyrin + light, resulting in destruction of the primary tumor. Schwartz was empirically examining various porphyrins with regard to their tumor-localizing properties and eventually hit on a process that yielded a superior product. This involved dissolving crude hematoporphyrin in a mixture of sulfuric + acetic acids, after which the water-insoluble product was dissolved in dilute base. This was achieved by a strictly empirical process, but it did produce a very effective product. It was named HPD (hematoporphyrin derivative) since the investigators had no idea as to the structure. Schwartz, his clinical collaborator Robert Lipson and the pathologist James Winkelman have published accounts of their early studies [5].

While HPD could be successfully used for tumor localization and therapy, it was important to elucidate the structure so as to satisfy the requirements of the FDA. We discovered that it was feasible to separate the active material from the much less active porphyrin monomers on an LH-20 column: a Sephadex-like material that can be used in non-aqueous solvents [6]. A mixture of porphyrin dimers and higher oligomers was eluted well-separated from everything else, and could be characterized with regard to photophysical and photosensitizing properties.

My group later turned to more 'photobiological' work. This initially involved an examination of structure-activity relationships, characterizing a wide variety of photosensitizing agents with regard to their affinity for sub-cellular organelles [7]. This turned out to be quite useful in the later examination of mechanisms of photokilling. Studies on the use of ultrasound for porphyrin activation were initiated with Charles Cain at the University of Michigan [8] and with surgical applications of protoporphyrin derived from amino-levulinic acid and the chlorin derivative NPe6 with Drs. David Fromm and John Webber in our Department of Surgery [9]. Studies on atheromas were done with Kathryn Woodburn at Pharmacyclics Inc, Sunnyvale, CA [10].

In many of our mechanistic studies, we have used the mouse leukemia L1210 cell line. This line is 'primed' for apoptosis, with genomic DNA degradation detected within 3-10 min after irradiation [11,12]. While this can provide an estimate of phototoxic events, it is necessary to

examine these in a variety of other malignant and some normal cell types to assess the relevance of L1210 studies.

A major breakthrough occurred in 1999 when we discovered that a prime target for PDT, involving mitochondrial or endoplasmic reticulum (ER) targets, was the anti-apoptotic protein Bcl-2 [13]. This accounted for the rapid initiation of apoptosis in a variety of different cell lines. This initial study was soon confirmed by others, notably by Oleinick's group at Case Western Reserve. She reported that PDT could affect both Bcl-2 and a related protein termed Bcl-x_L, in adhering cell lines. In cells grown in suspension, Bcl-x_L is spared, something we later traced to the discovery that the latter protein tends to be cytosolic in suspension lines, and membrane-bound in adhering cells [14]. Since the photosensitizing agents our groups were using were sufficiently hydrophobic to be rapidly bound to cell membranes, cytosolic proteins are generally spared from photodamage.

In collaboration with Prof. John Reiners Jr at Wayne State University, we found that photosensitizing agents that concentrate in lysosomes fail to affect Bcl-2, but cause lysosomal photodamage instead, resulting in the release of proteases into the cytosol. The action of these enzymes results in a cleavage of the pro-apoptotic protein Bid, resulting in the formation of a truncated version (t-Bid). This species can insert into the mitochondrial membrane, causing a release of cytochrome c, leading to the initiation of apoptosis [15].

Later, we began to study the role of autophagy in PDT. This was prompted by findings that both early- and late-stage responses to PDT often involved the appearance of cytosolic vacuoles that later turned out to represent autophagosomes. Autophagy is normally a process active during periods of starvation, when cells recycle their components by forming small vacuoles (autophagosomes) that engulf components to be digested. These vacuoles next fuse with lysosomes that release proteases. The net result is the conversion of cytosol and some organelles to their constituent amino acids and other small molecules that can be re-utilized. In the context of PDT, it appears that photodamaged mitochondria, ER and other cell components can thereby be recycled; perhaps before release of cytochrome c results in an apoptotic response. A wave of autophagy occurs soon after irradiation of photosensitized cells, followed by apoptosis and cell death, with some cells also dying by an excess of autophagy as they attempt to recycle too much of their contents. In cells with a critical autophagy gene suppressed, we found that there was an enhanced response to low-dose PDT, indicating that autophagy plays a role in the 'shoulder' on the dose-response curve [16-18].

The ability of autophagy to offer protection from the phototoxic effects of PDT is likely to be a function of the very steep dose-response curve. If a critical level of Bcl-2 photodamage is exceeded, apoptosis is markedly promoted and since this is an auto-catalytic process, irreversible effects on viability rapidly ensue. This phenomenon is also demonstrated by the promotion of PDT photokilling by small-molecule antagonists of Bcl-2 function [19]. Even an LD₁₀ level of such antagonists (*i.e.*, a level that reduces viability by only 10%) can turn an LD₃₀ PDT dose into an LD₉₀ dose.

Our most recent project involves the examination of the role of reactive oxygen species (ROS) on phototoxicity. It has been generally assumed that the major cytotoxic product formed during the irradiation of a photosensitizer is singlet molecular oxygen. This is a highly-reactive species that will oxidize the first biological molecule it encounters. As a result, only photosensitized cells that are irradiated will succumb to photodamage, since singlet oxygen is too reactive to migrate into the circulation. Other ROS are also formed during irradiation, including superoxide anion radical, hydrogen peroxide and hydroxyl radicals. Depending on the cell line and the concentration of detoxifying enzymes, some of these species can also play a role in photodamage.

The Future of PDT

In addition to continued studies into modes of photokilling, my role in the field involves the organization of a yearly conference sponsored by SPIE, a society associated with biomedical engineering. SPIE holds a variety of conferences at different world-wide sites, including one in California every January. I will also organize the next Congress of the International Photodynamic Association in Seattle, June 2009. Together with other conferences, both clinical and multi-disciplinary, these meetings aid in the exchange of information and providing avenues for the reporting of new research results.

The future of the field depends not only on the holding of conferences, but also on efforts to bring new people into the picture. With the NIH funding levels in the 12th percentile range, this gets increasingly difficult. The pathway that I followed, leading to over 50 years of NIH support, has largely vanished. The optimistic outlook is that new people will be able to build on what has gone before and find new approaches to the treatment of cancer, and perhaps of microbial infections and other medical problems, based on selective photosensitization.

Acknowledgments

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