Photochemistry and Photobiology, 2019, 95: 460–461

Highlight Article (Invited)

Secondary Dark Reactions Following Photodynamic Treatment are More Damaging Than Previously Thought†

Callistus Chiemezie[1,2] and Alexander Greer*[1,2]

[1]Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, NY
[2]Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, NY

Received 1 July 2018, accepted 24 July 2018, DOI: 10.1111/php.12984

ABSTRACT

Photodynamic treatment is often thought to produce reactive oxygen species (ROS) that directly induce killing; the nomenclature and phrases revolve around such notions of light-dependency. Few studies reference the possible existence of oxidation products formed in secondary reactions, which bear cytotoxicity competitive to their ROS precursors. Here, we highlight the paper by Girotti and Korytowski in this issue of *Photochemistry and Photobiology*, which does just that. In this paper, they report on cholesterol hydroperoxides, which are formed after photosensitized oxidation and yield cytotoxic mixtures in dark reactions after the light's turned off. Some of the hydroperoxides are transported by protein carriers and damage tissue outside their site of origin. A similar dark cytotoxicity may be anticipated for biological peroxides from *in vivo* photodynamic therapy.

COMMENTARY

Deciphering downstream reactions following photodynamic treatment can be problematic. The detailed cholesterol photooxidation studies conducted by Girotti and Korytowski (1) provide this insight into these very reactions.

Their studies reported in *Photochemistry & Photobiology* (1) describe the fate of cholesterol hydroperoxides produced in sensitized photooxidation. That fate provides key insight for the secondary reactions, such as the cholesterol hydroperoxides dark decomposition, which can be more cytotoxic than the primary reactions. Depending on the presence of metals and reagent concentrations, these dark reactions can lead to a chain process competitive in toxicity than the photo-generated precursors (Fig. 1). Chemists have long known that rate constants vary widely in reactions of ROS, and the lifetimes of initially formed peroxides depend on their structure and surrounding medium (2–4). Given the potential of formed peroxides to have cytotoxicity, new findings could improve understanding of light and dark dependent reactions in a range of photodynamic treatments.

Complementary cytotoxicity

The article described improvements in understanding of the cytotoxicity of photodynamic treatment of lipids. It is a two step process: (1) an "ene" reaction of $^1O_2$ leading to 5α–OOH, 6α–OOH, and 6β–OOH, as well as type I H-atom abstraction at the C7 position leading to 7α–OOH and 7β–OOH. Then, (2) downstream dark reactions take place and the cytotoxic hydroperoxide products deal their damage. The slow degrading 5α–OOH is more cytotoxic, whereas the fast degrading 7α–OOH and 7β–OOH are less cytotoxic. Attributing these post irradiation dark cytotoxic effects requires work. It is particularly interesting how the authors successfully segregated primary light from secondary dark events to help understand how photodynamic effects arise in time.

A lot of information on the light-to-dark process can be lost because of analytical difficulties. Few chemical probes are reliable over the light-to-dark process and can fail when the lights are turned off. However, Girotti's cholesterol probes show great versatility as, 5α–OOH probes for $^1O_2$, 7α–OOH and 7β–OOH probes for free radicals, while their subsequent decomposition can be traced forming epoxides and alcohols. In other words, the cholesterol probe allows reliable measures of type I and type II photosensitization mechanisms, and dark decomposition processes, requiring only a HPLC instrument. While other probe molecules also qualify, such as polyols and anthracenes, they often lack the ability to intercalate membranes for unambiguous evidence of the light and dark process.

Taken together, the light and dark variables reveal new insight into oxidation mechanisms. A major goal of PDT research is to be able to generate species whose cell killing ability can be controlled. Less commonly mentioned is the fact that $^1O_2$ can add to compounds and form perepoxy, zwitterionic and persulfoxy intermediates, which in dark processes are more powerful oxidants than photogenerated $^1O_2$ (5,6). Figure 1 shows that to form 5α–OOH, $^1O_2$ avoids the bulky C6 methyl, which accounts for minor amounts of 6α–OOH and 6β–OOH. The existence of 7α–OOH and 7β–OOH can be rationalized in terms of a type I reaction and stepwise addition of $^3O_2$ to an allylic radical site. However, control reactions revealed the fact that allylic isomerization of 5α–OOH to 7α–OOH or other shuffling reactions are slow and are not a concern in deducing the primary and secondary reaction events. The results of Girotti *et al.* have important implications for separating the primary and secondary reaction events in photodynamic action.

*Corresponding author email: agreer@brooklyn.cuny.edu (Alexander Greer)
†This article is part of a Special Issue celebrating Photochemistry and Photobiology's 55th Anniversary.

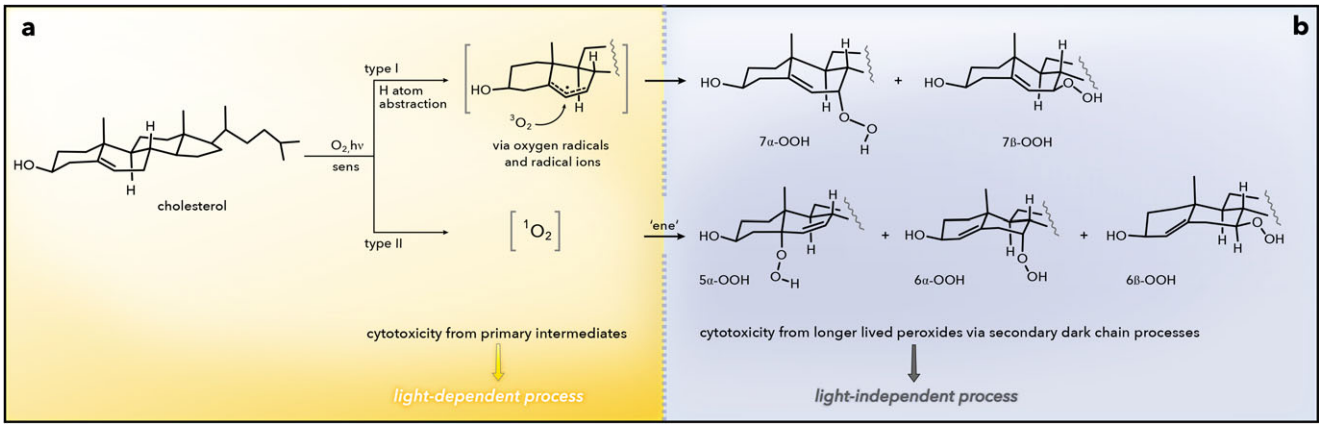

Figure 1. Complementary insight into cytotoxicity derived from the photosensitized peroxidation process, and the peroxide decomposition process. (a) The first step is light-dependent; the excited sensitizer produces cholesterol hydroperoxides from oxygen radicals or radical ions (type I) and singlet oxygen (type II) (Ref. 7). These cholesterol hydroperoxides can be stable for periods of time. (b) The second step is *light-independent*; the cholesterol hydroperoxides in turn decompose thermally by a dark reaction. In some cases, this decomposition is catalyzed by metals, and in other cases, it is by enzyme detoxification. The hydroperoxides have longer lifetimes if left to decompose on their own volition or if shielded by a protein. Nonetheless, the cholesterol hydroperoxides exhibit a cytotoxicity distinguished separately from the ROS by photoreactions in (a).

It is striking that peroxide products can be thought of as toxins comparable to ROS often thought to kill after photodynamic treatment. The work of Girotti *et al.* (1) reveals a key point, that may be viewed as like a one two punch, that is, light-dependent/dark-dependent processes that occur one after another. Such initial light and subsequent dark processes can account for deeper understanding of and improve our control of photodynamic effects. Such endeavors could make PDT applications more effective, so long as light-dependent and light-independent processes continue to be even better deciphered.

Acknowledgements—We acknowledge support from the National Science Foundation (CHE-1464975). We thank Sarah J. Belh for comments and Leda Lee for the graphic arts work.

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