Reprinted from *American Biotechnology Laboratory* September 2001

Image analysis used to facilitate cancer research

BY RON GOLDMAN, ROBERT BISSONNETTE, AND SORAYA SHARFAEI

ALTHOUGH TRADITIONAL cancer treatments such as surgery, radiotherapy, and chemotherapy have made significant advancements over the past century, emerging therapies are playing an increasingly important role in the fight against one of the world's leading causes of death. One such approach is photodynamic therapy (PDT). A fairly new method of treatment, PDT combines light and photosensitizing drugs to cause cell death and tumor destruction.

When light-sensitive molecules referred to as photosensitizers are activated by light at predetermined wavelengths, energy is transferred to surrounding oxygen molecules that produce highly reactive compounds comprising mainly singlet oxygen. These toxic molecules in turn damage cell membranes, organelles, nucleic acids, and other vital cell components, causing cell death.[1]

The technique has been used to treat numerous types of cancers including lung, esophagus, skin, bladder, and brain, and benign diseases such as menorrhagia, psoriasis, and macular degeneration.[2] At the University of Montreal Hospital Centre (Montreal, Quebec, Canada), one research project addressed the use of PDT in skin cancers. This article is a summary of the experiments published in full in the *British Journal of Dermatology.*[3] It explains how image analysis helped design experiments in which low-level systemic PDT with 5-aminolevulinic acid (ALA) could be useful in the prevention or delay of photocarcinogenesis in hairless mice exposed to ultraviolet (UV) radiation.

Experimental procedures

Hairless mice were sacrificed 3 hr after a 40-mg/kg intraperitoneal injection of ALA. Four skin specimens of UV-exposed back skin and non-UV-exposed ventral skin, embedded with small- and medium-size tumors, were placed in a frozen section medium and cooled in liquid nitrogen.

Subsequent to freezing, five 10-µm-thick sections from each sample were cut with a cryostat and stored at –70 °C. Tissue sections were then prepared and imaged using minimal light in order to avoid photobleaching of protoporphyrin IX (PpIX). To identify the localization

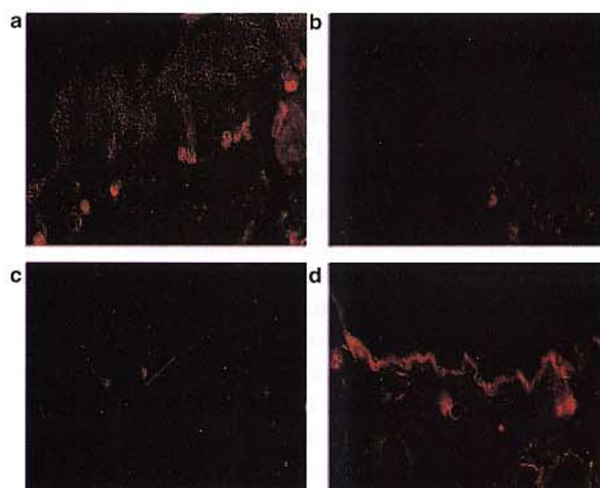

Figure 1 *Protoporphyrin IX fluorescence 3 hr after IP injection of ALA at 40 mg/kg in normal skin and in small tumor. Fluorescence microscopy of normal skin (c, d) and small tumor (a, b) at 40 min (a, c) and 3 hr (b, d) after IP ALA at 40 mg/kg.*

of PpIX, fluorescence sections were fixed in acetone and stained with toluidine blue.

Fluorescence images of sections were generated using an Optiphot-2 fluorescence microscope (**Nikon,** Tokyo, Japan) equipped with a DC 330E thermoelectrically cooled charge-coupled device (CCD) camera (**Dade,** Michigan City, IN) connected to a Kayak personal computer (**Hewlett-Packard,** Palo Alto, CA). Fluorescence recording of images was made possible with the use of a filter block containing a 480-nm shortpass excitation filter and a 635 ±10 nm bandpass emission filter. In order to avoid a photobleaching effect, integration times of 1 sec were used to capture images (*Figure 1*).

Following fluorescence recording, slides were stained with toluidine blue without moving them from their original position on the microscope stage. After staining, images of the same areas were recorded to confirm their histology. With the use of Vision image analysis software, version 3.0 (**Clemex,** Longueuil, Quebec, Canada), images were captured and analyzed. The software was used specifically to measure the fluo-

rescence intensity per unit area of the epidermis and tumoral areas.

Discussion and conclusion

ALA is not a photosensitizer in itself. During the first stage of the heme biosynthetic pathway, ALA is formed from glycine and succinyl-CoA by ALA synthetase. The last stage incorporates iron into PpIX and is under the control of the enzyme ferrochelatase. The exogenous administration of ALA leads to PpIX intracellular accumulation, which is a photodynamically active and highly fluorescent compound.

In some tumors, it appeared that the action of the rate-limiting enzyme ferrochelatase is lower, causing selective accumulation of PpIX in cancer cells.

Since ALA induces preferential localization or retention of protoporphyrin in malignant tissues, it might prevent the appearance of clinically visible skin cancers by the elimination of individual or microscopic accumulation of cancer cells. The treatment of actinic keratoses with ALA-PDT can be construed as a chemoprevention modality since actinic keratoses are precursors of squamous cell carcinoma. The authors hypothesized that repeated PDT could destroy microscopic skin cancers before they evolve into clinically apparent tumors and therefore prevent the development of visible skin cancers. Consequently, skin cancer development may be optimally delayed if PDT is performed when the photosensitizer fluorescence ratio of the malignant to the normal epidermis is the highest.

In these experiments, image analysis was able to determine the time at which the fluorescence intensity ratio of tumor versus normal skin was the highest.

References

1. Levy JG. Photodynamic therapy: using light to treat cancer. Trends Biotechnol 1995; 13:14–7.
2. Illingworth J. Cancer treatment and photodynamic therapy: opportunities and limitations. www.bmb.leeds.ac.uk/illingworth/bioc3380/pdt.htm.
3. Sharfaei S, Viau G, Lui H, Bouffard D, Bissonnette R. Systematic photodynamic therapy with aminolevulinic acid delays the appearance of UV-induced skin tumors in hairless mice. Brit J Dermatol 2001; 11:1–9.

Mr. Goldman is Marketing Director, ***Clemex Technologies, Inc.,*** *800 Guimond, Longueuil, Quebec, Canada, J4G 1T5; tel.: 450-651-6573; fax: 450-651-9304. Dr. Bissonnette and Dr. Sharfaei are with the Division of Dermatology and Department of Pathology, University of Montreal Hospital Centre, Montreal, Canada.*