# Oxygen Levels in Normal and Previously Irradiated Human Skin as Assessed by EF5 Binding

Sydney M. Evans[1], Amy E. Schrlau[1], Ara A. Chalian[2], Paul Zhang[3] and Cameron J. Koch[1]

The oxygen status of skin is a controversial topic. Skin is radiosensitive, suggesting it is well-oxygenated. However, it can be further sensitized with nitroimidazole drugs, implying that it is partially hypoxic. Skin oxygen levels are difficult to measure with either electrodes or the hypoxia-monitoring agent $^{3}$H-misonidazole. For the latter, binding has previously been reported to be high in murine skin, but this could be attributed to either non-oxygen-dependent variations in nitroreductase activity, drug metabolism, and/or actual oxygen gradients. We obtained tumor and skin from patients given EF5, a 2-nitroimidazole tissue hypoxia monitor. We performed immunohistochemical studies using highly specific monoclonal antibodies for the hypoxia-dependent production of EF5 tissue adducts. Some tissue sections were counterstained using either Ki67 for proliferation or CD31 for vessels. We found that the human dermis is well-oxygenated, the epidermis is modestly hypoxic and portions of some sebaceous glands and hair follicles are moderately to severely hypoxic. Normal and irradiated skin had similar oxygenation patterns. Control studies demonstrated that these observations are not due to tissue variations in nitroreductase activity. The importance of the highly heterogeneous distribution of oxygen in skin requires further study, but recent investigations suggest that skin hypoxia may have important clinical ramifications including mediating cellular transformation.

*Journal of Investigative Dermatology* (2006) **126,** 2596–2606. doi:10.1038/sj.jid.5700451; published online 29 June 2006

## INTRODUCTION

The oxygen level in human skin is of considerable interest to scientists and physicians in a diverse range of fields. Anesthesiologists are interested in skin oxygenation as a monitor of systemic oxygenation. Wound healing or its absence is known to be affected by tissue oxygenation (Hunt *et al.*, 2004) and this is of concern to surgeons. Radiation oncologists have long known that tissue oxygenation affects the extent of radiation damage; the skin overlying irradiated volumes is at substantial risk for complications during radiation treatment of superficial tumors, for example breast cancer. Acute radiation side effects include erythema and desquamation; hair loss occurs during or shortly after therapy. Late radiation effects include fibrosis, telangiectasia, and secondary malignancies. The etiology of the late radiation effects is not well defined, especially related to oxygen dependence. However, there are data to suggest that long-term production of reactive oxygen species within the irradiated site may be important (Moeller *et al.*, 2004). The efficacy of other cancer treatment modalities such as chemotherapy and photodynamic therapy is also known to be modified by tissue oxygenation (Yamagata *et al.*, 1992; Busch *et al.*, 2002).

There have been numerous oxygen partial pressure ($pO_2$) studies of skin in animal models, particularly rodents and pigs. However, the direct measurement of $pO_2$ in unperturbed human skin has been limited by the lack of appropriate methodology. Electrode and other studies have emphasized the $pO_2$ in wounded skin and confirmed the presence of hypoxia in this situation (Koch *et al.*, 1997; Hunt *et al.*, 2004). Needle electrode measurements in normal skin are difficult because of the skin's highly heterogeneous composition, its thinness in mice, and its toughness in all mammals. Indirect information on the $pO_2$ of human skin comes from its response during radiation therapy. The skin readily manifests acute radiation damage, inferring that the proliferating components of the skin are well-oxygenated. This is also true for hair production and growth, as hair regrowth is limited following radiation treatment. Extensive studies by Cobb *et al.* have documented the uptake of the prototypic metabolic hypoxia detector $^{3}$H-misonidazole in epidermis and various appendages of rodent skin (Cobb and Nolan, 1989; Cobb *et al.*, 1989, 1990b). However, the conclusion reached in these studies was that this and similar binding in other ''aerobic'' rodent tissues was artifactual due to high nitroreductase activity (Cobb *et al.*, 1990c). In contrast, *in vivo* studies from other laboratories showed that high misonidazole binding in esophagus could be inhibited

[1]*Department of Radiation Oncology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA;* [2]*Department of Otorhinolaryngology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA and* [3]*Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA*

*Correspondence: Dr Sydney M. Evans, Department of Radiation Oncology, University of Pennsylvania School of Medicine, 195 John Morgan Building, Philadelphia, Pennsylvania 19104, USA.*
*E-mail: sydevans@mail.med.upenn.edu*

*Abbreviations: AUC, area under the curve; CRB, cube reference binding; $pO_2$, oxygen partial pressure*

*Received 3 January 2006; revised 23 March 2006; accepted 13 April 2006; published online 29 June 2006*

by 95% oxygen breathing (Parliament *et al.*, 1992). More recently, $pO_2$ values of $<5$ mm Hg have been recorded in mouse dermis using electron paramagnetic resonance techniques (Krzic *et al.*, 2001). Thus, the question of the actual $pO_2$ of skin remains unresolved for both animals and humans.

EF5 is a 2-nitroimidazole drug that was specifically designed to monitor tissue hypoxia. It has been validated for quantitative studies of both neoplastic and non-neoplastic tissue $pO_2$ in humans and in animal models (Koch *et al.*, 1995, 2001; Evans *et al.*, 1996, 2004b, 2006; Bergeron *et al.*, 1999; Koch, 2001). Herein we present data based on EF5 binding as a measure of tissue oxygenation in irradiated and untreated human skin. Our findings support the extensive literature on the radiation sensitivity of the skin because we find that the majority of the skin, that is, the dermis is oxic. However, EF5 binding is present to varying degrees in human epidermis and other skin appendages, including portions of some hair follicles and sebaceous glands. In order to determine whether this heterogeneity was oxygen dependent *versus* an artifact of nitroreductase activity or nonspecific binding, we performed control studies to assess the maximum tissue binding under conditions of severe oxygen deprivation. These data were obtained by incubating small tissue cubes under 0.02% oxygen with EF3, a sister compound of EF5. This procedure is called the ''cube reference binding'' (CRB) study (Koch, 2001; Evans *et al.*, 2004b). We hypothesize that all skin appendages in a given patient will bind EF3 equally under the CRB condition and, therefore, any variations of binding *in vivo* would reflect true oxygen differences. The resultant data are consistent with the conclusion that gradients of EF5 binding result from gradients in oxygen tension rather than variations in nitroreductase activity. However, the latter contributes substantially to patient-to-patient variability in absolute binding and, therefore, must be taken into account in order to obtain a reasonable estimate of actual tissue $pO_2$.

## RESULTS

Skin and tumor tissues from 19 patients were assessed. Seventeen patients were receiving surgery for head and neck cancer and two were being treated for extremity sarcoma (Table 1). Ten patients had received radiation therapy preceding the surgery at which the tumor and skin tissue were removed. Skin from two patients who did not receive EF5 was available for control studies of CRB (see below for methodological and analytic methods).

**Table 1. Patient information**

| PT ID no. | Sex | Age (years) | Race | Previous radiation therapy | Months[1] | Tumor site | Skin type studied |
|---|---|---|---|---|---|---|---|
| 66 | M | 47 | C | Yes | 3 | H&N | Normal and XRT |
| 67 | M | 62 | C | No | 0 | H&N | Normal |
| 73 | M | 65 | C | Yes | 7.5 | H&N | XRT only |
| 75 | M | 48 | C | Yes | 24 | H&N | XRT only |
| 79 | F | 60 | C | Yes | 10 | H&N | Normal and XRT |
| 80 | M | 45 | C | No | 0 | H&N | Normal |
| 81 | M | 71 | C | No | 0 | H&N | Normal |
| 83 | M | 70 | C | No | 0 | H&N | Normal |
| 85 | M | 57 | C | No | 0 | H&N | Normal |
| 91 | M | 63 | C | Yes | 3 | Sarc | XRT only |
| 92 | M | 66 | A | Yes | 1 | Sarc | XRT only |
| 93 | M | 63 | C | Yes | 4 | H&N | Normal and XRT |
| 94 | M | 60 | C | Yes | 8.5 | H&N | Normal and XRT |
| 95 | F | 59 | C | Yes | 9 | H&N | XRT only |
| 97 | M | 74 | C | No | 0 | H&N | Normal |
| 98 | M | 64 | C | Yes | 18 | H&N | XRT only |
| 102 | M | 69 | C | No | 0 | H&N | Normal |
| 103 | F | 62 | C | No | 0 | H&N | Normal |
| 104 | M | 68 | C | No | 0 | H&N | Normal |
| 105[2] | M | 65 | C | — | — | — | — |
| 106[2] | F | 54 | C | — | — | — | — |

A, Asian; C, Caucasian; H&N, head and neck cancers; Sarc, sarcoma; XRT, radiation therapy.
[1]From end of XRT to biopsy.
[2]CRB studies only.