IMAGING HYPOXIA IN DISEASED TISSUES

S. M. Evans,1 M. Bergeron,2 D. M. Ferriero,2 F. R. Sharp,2 H. Hermeking,3
R. N. Kitsis,4 D. L. Geenen,4 S. Bialik,7 E. M. Lord,5 and C. J. Koch6

1Department of Clinical Studies
School of Veterinary Medicine
University of Pennsylvania
2Department of Neurology
University of California
San Francisco, California
3Molecular Genetics Laboratory
Johns Hopkins University
Baltimore, Maryland
4Department of Cardiology
Albert Einstein College of Medicine
Bronx, New York
5Cancer Center
University of Rochester
Rochester, New York
6Department of Radiation Oncology
School of Medicine
Philadelphia, Pennsylvania

INTRODUCTION

Hypoxia is a physiologic condition which is a component of many disease processes. Hypoxia can be mediated by low inspired oxygen, lung disease or organ ischemia. Although, in general, the delivery of oxygen to tissue has sufficient excess capacity to compensate for a wide range of demand (luxury perfusion), a marked deficiency of intracellular oxygen results in immediate tissue dysfunction. Prolonged hypoxia is the prevalent cause of irreversible injury in all mammalian tissues (Cotran et al., 1989). Hypoxia plays a role in the pathogenesis of stroke (for review see Hossmann, 1994), cardiac disease (Rumsey et al., 1994), cancer (for review see Adams, 1990), ocular disease (for example see Flammer, 1994), renal disease (Brezip and Rosen, 1995), rheumatoid arthritis (for review see Edmonds et al., 1995) and wound healing (for review see Hunt, 1988), among others. In many diseases, including cancer, the presence of hypoxia decreases the effectiveness of therapy and signals a poor prognosis (Brizel et al., 1996; Hockel et al.,
Potential markers that could be used to estimate tissue oxygen levels include blood vascular perfusion, acidosis (due to the production of lactate in ischemic tissue) or a direct marker of cellular pO₂. Positron emission tomographic (PET) and magnetic imaging spectroscopic (MRS) techniques have been developed to measure intracellular pH (Buxton et al., 1987; McCoy et al., 1995). Unfortunately, these techniques are complex and difficult to perform as routine procedures. In tumors, the highly variable blood flow results in a wide range of intracellular pH measurements. In any tissue, the measurement of pH is an indirect indicator of tissue hypoxia.

Blood vascular perfusion imaging depicts the delivery of plasma to the tissues; this measurement may not directly correspond to the delivery of oxygen to tissues. In tumor tissues, for example, elegant studies using a “window chamber model” have demonstrated the presence of plasma flow without red cells and flow in vessels which are devoid of oxygen (Secomb et al., 1993). Similarly, perfusion measurement do not address whether oxygen delivery is sufficient to meet the needs of the regional tissues, i.e. non-transmural cardiac infarction. None the less, many techniques have been developed to measure tissue perfusion, either directly or indirectly. Experimental techniques for measuring blood flow include direct measurements of blood flow using isolated organs or tumors (Evans, 1994) and ex vivo techniques (i.e. Hoechst dyes; see Koch et al, this conference). Clinically relevant techniques include Doppler blood flow, and washin or washout techniques. The regional distribution of radionuclide tracers (such as 99mTc-hexamethylpropylene amine oxime “HPOA” or thallium) have been used to study cerebral and cardiac ischemic disease. Although these methods can identify regions of low flow, they cannot differentiate between scar, functional parenchymal tissue, ischemia or necrosis.

An imaging agent or technique that specifically identifies hypoxic, viable (although perhaps dysfunctional) tissue is needed. Nitroimidazole agents are ideal for this function because they are preferentially bound in hypoxic viable tissues (first described by Varghese et al., 1976) but cannot be metabolized by regions of tissue necrosis. EF5 is a 2-nitroimidazole drug which binds to protein sulphydryls in hypoxic cells (Lord et al., 1993; Koch et al., 1995). We have previously demonstrated that the adducts thus formed can be localized using specific monoclonal antibodies conjugated to the fluorescent dye, Cy3 (Lord et al., 1993). Using immunohistochemical imaging techniques, regions of hypoxia can be identified as areas of binding. Non-binding regions are either oxic tissues or necrotic tissues which cannot metabolize the EF5. Oxic viable tissues can be differentiated from necrotic tissues based upon re-staining of slides with hematoxalin and eosin or Hoechst 33342, which binds to intact DNA. One of the strengths of the EF5 binding technique is the large dynamic range of binding and the resultant ability to demonstrate a continuum of cellular pO₂ levels. This was first demonstrated using flow cytometric analyses (Koch, 1995) where the binding of 9L tumor cells incubated in vitro in 4% O₂ vs. N₂ varied by a factor of 50; moderate O₂ levels (1.2% O₂) show intermediate levels of EF5 binding. Using histogram analyses of immunohistochemical staining of tissue sections, this continuum of binding corresponding to cellular pO₂ can also be demonstrated (Laughlin, 1996).

The purpose of this paper is to demonstrate the wide range of applications of the EF5 technology in several disease processes: cancer, stroke and myocardial ischemia. Currently, the analysis of the presence and distribution of hypoxia by EF5 binding requires the acquisition of tissue for either immunohistochemistry or flow cytometric analyses. For analysis of hypoxia in neoplastic tissues, this is entirely appropriate because