Detection of Hypoxic Cells by Monoclonal Antibody Recognizing 2-Nitroimidazole Adducts

Edith M. Lord, Lee Harwell, and Cameron J. Koch


ABSTRACT

Hypoxic cells in tissue pose many medical problems, and there is a need for more accurate measurements of tissue hypoxia. However, measurement of the pO2 and the extent of hypoxia within normal and tumor tissue have proven difficult. One of the most sensitive of the currently available methodologies involves the oxygen-dependent metabolic activation of nitroheterocyclic drugs, leading to adducts between the drugs and cellular macromolecules. Limitations of the present methods and adduct-detection methods prompted the present studies. A pentafluorinated derivative [EFS; 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamide] of etanidazole was synthesized with the expectation of lessening the problems and limitations of etanidazole. ESF-protein conjugates, prepared by radiochemical reduction, were found to be immunogenic and allowed the development of monoclonal antibodies. One of these antibodies, ELK2-4, has been characterized and found to be highly specific for the ESF adducts under all conditions of storage, handling, and activation. An additional antibody to the ESF adducts was prepared. Antigen expression was localized in tumor cells, tumor xenografts, and tumor biopsies by immunohistology using fluorochrome-conjugated secondary antibodies. The latter can be used for imaging techniques in vivo.

INTRODUCTION

Oxygen consumption by cells provides their major source of energy, through oxidative phosphorylation. Limitations in oxygen supply from the tissue vasculature result in hypoxia. Hypoxia can occur rapidly, during the shutdown of blood flow from stroke or traumatic injury, or much more slowly in cases which arise from various other types of progressive vascular disease. Alternatively, the rapid growth of some tumors can outpace their host-provided blood supply causing regional hypoxia and necrosis. In these and other situations, the hypoxic cells pose severe medical problems. For example, hypoxic tumor cells are radioresistant and can cause treatment failure. The same supply or diffusion barriers which prevent oxygen from reaching the hypoxic regions of tumors can prevent the diffusion of reactive chemotherapeutic drugs (1–3). Alternate therapies such as immunotherapy may be similarly limited by the impaired ability of cytotoxic host cells or antibodies to reach the hypoxic areas.

Detection of hypoxia in tissue is thus important in many areas of disease, with the most obvious involving two of the leading causes of death, cardiovascular disease and cancer; it has become clear that methods are required to identify hypoxia in individual patients. The presence of hypoxic cells in relatively large, previously diagnosed human tumors has been measured directly using polarographic oxygen needle electrodes (4–8) and has been inferred in numerous other ways (9, 10). Furthermore, direct comparisons of tumor hypoxia and radiotherapy resistance using needle oxygen sensors have shown a dramatic correlation (7). However, measurement of hypoxia in smaller tumors or regions of ischemia requires a detection system which can, in principle, monitor very low oxygen concentrations in small, inaccessible tissue volumes. The range of oxygen concentrations which cause radiation or chemical resistance is greatly below the venous pO2 of about 35 torr (mm Hg; see Fig. 1). Measurements of such low oxygen values are technically challenging for two key reasons. The first is that many measurement technologies have only a limited dynamic range, sometimes above and sometimes below the range of interest (Fig. 1). For example, changes in pyridine nucleotide fluorescence (11) are most sensitive at oxygen levels greatly below those which affect radiation damage, whereas changes in hemoglobin saturation occur at oxygen levels substantially above those which affect radiation sensitivity. One exception involves the oxygen dependence of radiation damage to DNA, which is thought to closely parallel that for survival (12). At present, technical limitations can sometimes prevent polarographic needle sensors from accurately monitoring the very low levels of oxygen characteristic of radiation resistance (8). Macroscopic Clark sensors (13) and phosphorescence decay techniques (14) can adequately monitor oxygen over the entire range of oxygen concentrations shown but in any given application are limited to a dynamic range of about 1000. Only the latter can be used for imaging techniques in vivo. One of the newest monitoring techniques under current development is electron paramagnetic resonance (15). This technique may have the potential for an appropriate dynamic range, but elimination of interactions of the probes with biological materials is difficult.

The second technical limitation involves the nature of the oxygen-dependent signal. True oxygen monitors, like polarographic oxygen sensors (irrespective of other design factors), have a signal which is proportional to the measured quantity (oxygen partial pressure) so they can become noisy and inaccurate at such low levels (13). Furthermore, they tend to average oxygen levels over relatively large tissue volumes. Thus measurement of a small region of extreme low oxygen levels and therefore limits detection.

What is required is a “no oxygen” detector. The signal from such a detector would be maximal in the absence of oxygen and would be inhibited by oxygen over the range of oxygen concentrations affecting the phenomena of interest. Examples include phosphorescence decay and the technique used in this report, nitroheterocyclic binding. It has been clearly shown that the metabolism of nitroheterocyclic drugs (RNO2), known as hypoxic cell-radiation sensitizing agents or radiosensitizers, leads to the formation of stable adducts with cellular macromolecules (“binding”) and that these adducts are formed at a much greater rate in hypoxic than in aerobic cells (1, 16–22). Thus, detection of these bound adducts can provide information on the relative oxygenation of tissue at a cell-to-cell resolution.

Received 5/24/93; accepted 9/30/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Work partially supported by Grant CA28332 (E. M. L.) from the National Cancer Institute and Grant DHP-27B (C. J. K.) from the American Cancer Society.

2 To whom requests for reprints should be addressed.
Several detection modalities exist for the nitroaromatic adducts: (a) for monitoring in situ, incorporation of radioactive or other isotopes with PET (23), single photon emission computed tomography (24), and MRI or MRS (25, 26); and (b) for monitoring via biopsy, β-emitting radioactive isotopes (22, 27), endogenous fluorescence of nitroheterocyclic products (21), and polyclonal antibodies against the adducts (28). If cellular adducts to nitroheterocyclics were accessible to antibodies provided by the blood supply, antibodies labeled with suitable isotopes would also provide an elegant in situ detection system.

Although polyclonal antibodies against adducts of a hexafluorinated derivative [CCl-103F; 1-(2-hydroxy-3-hexafluoroisopropoxy-propyl)-2-nitroimidazole] of the 2-nitroimidazole misonidazole have been made (28), all attempts at making MoAbs to adducts of this and other 2-nitroimidazoles have failed. MoAbs, techniques with their high specificity and tremendous variety of detection technologies, can provide the most detailed information on the cellular or tissue distribution of the adducts, including subcellular distribution of sites of metabolism and/or binding, or even the identification of adducts from individual proteins (Western blotting). An ideal detection scheme might include a water-soluble, fluorine-containing nitroheterocycle, allowing MRI/MRS or PET imaging of the actual drug or drug adducts, and a monoclonal antibody to the drug or drug adducts which allows all other detection possibilities. This report provides the first demonstration of such versatility in detection techniques.

MATERIALS AND METHODS

**Drug Synthesis.** A pentafluorinated derivative of etanidazole, EF5, appeared to have suitable properties based on some of our previous studies which have identified inconsistencies in the binding properties of several other 2-nitroimidazoles (29). This drug, in unlabeled and labeled form (2-14C position; 43 μCi/mg), was synthesized by Dr. M. Tracy and colleagues at Stanford Research International, Palo Alto, CA, and is referred to as EF5 in this manuscript. Synthesis and detailed characterization of this drug will be published elsewhere.

**Binding of EF5 to Cells in Tissue Culture under Defined Oxygen Conditions.** The cells used were derived from 9L rat glioma (30) or EMT6-Ro mouse mammary carcinoma (31). The cells were thawed from frozen stock on a roughly semiannual basis, and tests were made routinely to ensure that the cultures were free from *Mycobacteria* and other contaminants. The cells were cultured (37°C; 95% air + 5% carbon dioxide; 100% relative humidity) in the exponential phase of growth by twice-weekly transfers using Eagle’s minimal essential medium containing 12.5% v/v of either newborn calf serum (9L) or fetal calf serum (EMT6-Ro). Penicillin and streptomycin were also routinely included (all culture solutions were from Sigma Chemical Co.). On the day before an experiment, cells were trypsinized and plated onto glass Petri dishes; ~250,000 cells were confined to the central area of the dish followed by overnight incubation at 37°C as described previously (32). The dishes were then removed from the incubator, cooled to 0–4°C, and their medium was replaced with drug containing medium, first as a rinse (1 ml) which was simply aspirated and then as the actual medium used for the experiment (also 1 ml). Dishes were then placed in leak-proof aluminum chambers which were connected to a manifold, allowing them to be deoxygenated with a series of gas exchanges taking approximately 30 min. The confinement of cells to the central area of the dish and the use of a small volume of medium allow very rapid equilibration of the gas and liquid phase to improve the control of oxygen concentration (32). After gas exchange, the chambers were quickly warmed to 37°C by immersion in a water bath and then dried and transferred to a warm room, also at 37°C. To prevent minor gradients of oxygen or potentially larger gradients of nutrients/metabolites, the chambers were also shaken gently (1 Hz; 2.5-cm stroke).

**Binding of radioactive nitroheterocyclics after incubation under defined experimental conditions was assessed as described previously (33).**

Some cells were treated as above but nonradioactive EF5 was used instead. After incubation in air or nitrogen, the cells were removed from the glass dishes with trypsin and then allowed to attach to microscope slides where they were then stained using conventional immunohistochemical techniques (see below).

**Preparation of Antigen.** Protein conjugates of EF5 were prepared using radiochemical reduction methods in a two-step process. It had been shown previously (34) that the chosen protein must contain a high mol fraction of reduced cysteine residues for this process to be efficient, *i.e.*, to achieve a high fraction of (bound drug:reduced drug) and to achieve a density of adducts per protein which was as high as possible. The cysteine residues of most readily available proteins are either scarce, not accessible for adduct formation (*e.g.*, alcohol dehydrogenase; data not shown), or are oxidized as cystine dimers which are often important in determining the structure of the protein (*e.g.*, albumin). It was not possible to reduce protein cystines by the addition of excess quantities of reducing agents such as dithiothreitol or mercaptoethanol, which could simultaneously reduce and stabilize cystine-containing proteins. Drug-adducts would then preferentially form with the excess low molecular weight thiol, and separation of the proteins from the low molecular weight thiols inevitably led to large protein losses (data not shown). To circumvent this difficulty, we exploited the discovery that the cystine dimers of many proteins can be very efficiently reduced via a radiochemical chain reaction, *i.e.*, low doses of ionizing radiation in an oxygen-free, formate-containing solution (35). However, the modified protein is often relatively insoluble (possibly because of the formation of disulfide bridges between molecules). Thus it was necessary to identify a protein with high cystine content and having relative freedom from precipitation after radiochemical reduction. BBI (36), a trypsin/chymotrypsin inhibitor from soybeans (7 cystine bridges; molecular mass 7800), was found to have near optimal characteristics from this point of view, and reduction of up to an average of 8 cysteine residues was possible while maintaining protein solubility and thiol stability. The EFS-BBI conjugates were then made in a second radiochemical reduction step which could be accomplished in the same solution.

**Preparation of Monoclonal Antibodies.** C57Bl/cdJ × SJL/Br-H-2(k) mice were given injections of 15 μg of protein antigen emulsified with an equal volume of Freund’s complete adjuvant by i.m. injection at each of two

---

3 The abbreviations used are: PET, positron emission tomography; MRI, magnetic resonance imaging; CTS, magnetic resonance spectroscopy; MoAb, monoclonal antibody; EF5, pentafluorinated derivative of etanidazole; BBI, Bowman-Birk inhibitor; ELISA, enzyme-linked immunosorbent assay; AMCA, aminomethylcoumarin-acetic acid.

4 C. J. Koch, unpublished data.
was given i.p. 2 months later. Three weeks after the booster injection, a serum sample was tested for antibody activity against protein-EF5 adducts versus protein alone using a standard ELISA assay. A mouse whose antisera reacted with the EF5, whether it was conjugated to either the immunizing protein (BBI) or other proteins, was selected. This mouse received additional i.p. injections of EF5-BBI conjugate (80 μg) at 4, 3, 2, and 1 day before sacrifice and spleen removal. The fusion was performed as described previously (31). Polyethylene glycol (P7777; Sigma) was prepared at 35% in serum-free media containing 5% dimethyl sulfoxide (tissue culture grade; Sigma) with pH adjusted to 7.4 with 4 M NaOH. All cells and reagents were warmed to 37°C prior to the fusion. After the fusion, the cells were bulk-cultured in a roller bottle for 2 days. The cells were then centrifuged, resuspended in 10 ml of freezing medium (culture medium with 40% fetal bovine serum and 10% dimethyl sulfoxide), and frozen at 1 ml per vial.

One vial was subsequently thawed and cultured by splitting the cells into 5 96-well microtiter plates in 10% fetal bovine serum and HAT-containing culture medium (1.36 mg/ml hypoxanthine, 0.038 mg/ml aminopterin, and 0.0176 mg/ml thymidine) to select for fused cells, with 2000 irradiated mouse peritoneal cells/well as feeder cells. Wells were screened for specific antibody-producing cells using an ELISA assay and BBI versus BBI-EF5 conjugate as antigens. Six hybridomas were selected based on the differentiation of these two antigens and were further screened against a panel of additional antigens. One of these (ELK2-4) was selected based on its strong selective reaction against protein-EF5 conjugates and lack of reactivity against any other antigens tested, including whole-cell protein preparations. This clone was slowly adapted to growth in serum-free medium (EXCELL 300; JRH Biosciences), and relatively pure MoAbs were purified by a simple ultrafiltration using Amicon Centriprep ultrafilters (M, 30,000 cutoff). Some MoAb protein was made autofluorescent by the addition of AMCA using standard protein modification kits available from Pierce.

**Histological Staining.** Cells (9L; incubation at 37°C in the presence of 0.5 mM EF5 for 4 h in air or nitrogen) were allowed to attach to microscope slides. The slides were air dried, briefly exposed to a 0.1% solution of Triton X-100, and then rinsed to remove the detergent. The slides were treated overnight at 4°C with ELK2-4 antibody and rinsed for 10 min in phosphate-buffered saline. They were then stained with fluorescein-labeled goat anti-mouse IgG (The Jackson Laboratory), rinsed to remove unbound second antibody, and observed using a fluorescent microscope.

EMT6-Ro spheroids were grown as described previously and then placed in individual spinner flasks to allow development of an equilibrium fraction of hypoxic cells (37). EF5 was added at a final concentration of 0.5 mM and the spheroids were incubated at 37°C for 4 h. The spheroids were removed, washed, embedded in OCT compound, and frozen on dry ice. Cryostat sections (10 μm thick) were cut and placed on poly-l-lysine coated glass slides, and the sections were fixed in freshly prepared 1% paraformaldehyde for 10 min. After rinsing, blocking antiserum (2% normal goat serum) was added and sections were incubated for 30 min at room temperature. The slides were treated with ELK2-4 overnight at 4°C, washed 3 times, and then treated with fluorescein-labeled secondary antibody as indicated above. The slides were then washed and observed with a fluorescent microscope.

A BALB/c mouse (25 g) bearing an EMT6 tumor was injected i.p. with 1.2 ml of 2 mM EF5. Forty-eight h later, the mouse was sacrificed, and the tumor removed, embedded in OCT compound, and frozen on dry ice. Cryostat sections (~20 μm thick) were cut, and the sections were treated as above for the spheroid sections except that only one antibody was used, namely the AMCA-conjugated ELK2 described above. A similar experiment was performed in a Buffalo rat bearing a Morris 7777 hepatoma tumor, with the drug dose increased in proportion to the weight of the larger animal.

**RESULTS**

The oxygen dependency of binding was tested using 20 μM and 500 μM drug for EMT6-Ro cells in tissue culture. The rate of binding of EF5 in nitrogen was reduced 2-fold for cells at intermediate oxygen levels (~3 torr) and 10-fold for cells growing in 15 torr oxygen (Fig. 2). Note that at 37°C with gas phase in equilibrium with water vapor, the absolute oxygen concentration in micromolar is approximately 1.4 times the partial pressure in torr, where torr is the standard abbrevia-

![Fig. 2. Inhibition of binding by oxygen in EMT6-Ro cells. EFS concentrations were 500 μM (A, 1, 2) and 20 μM (3, 3, 2). Binding occurs at a much greater extent in hypoxic cells (O) than cells at intermediate oxygen levels (~3 torr or 0.4% O2), and a 15-fold reduction in binding is seen for cells growing in 15 torr (2%) oxygen (C). A log-log scale has been used to accommodate the large range of binding. Binding is linear with time, and the lines have been drawn to indicate this.](cancerres.aacjournals.org/pc5723/pc5723f2.jpg)

![Fig. 3. Competition ELISA assay using various nitroaromatic compounds to compete against EF5-protein adduct adsorbed onto ELISA plates. The compounds tested were EF5 (O), etanidazole (C), misonidazole (triangle), dimethylmisonidazole (square), NP-6 [2,4-dinitro-1H-pyrryl-1-yl(ethanol) (*)], and CCI-103F (■). Note that etanidazole did not compete with EF5 at 1000-fold excess concentration and that the most active competitor (CCI-103F) was at least 100-fold less reactive.](cancerres.aacjournals.org/pc5723/pc5723f3.jpg)
amines on the antibody. In the second, the modification is effected via protein sulfhydryl groups (HPDP); since antibodies do not have free sulfhydryl groups but contain a number of cystine disulfides, some of the latter were first reduced using mild radiochemical reduction (see "Materials and Methods"; Ref. 35). Addition of AMCA by either method did not alter the EF5 binding capacity of ELK2-4 antibody (Fig. 4).

In the above examples, the EF5-protein antigen used was derived by radiochemical reduction techniques. It was necessary to confirm that ELK2-4 antibody would also recognize macromolecular adducts of EF5 produced by cellular bioreduction pathways as would occur in vivo or in vitro. EMT6 tumor cells were pretreated with 0.5 mM EF5 under varying levels of oxygen or with etanidazole under extreme hypoxia. Protein extracts of whole cells were then made by homogenizing the cells in 0.1% Nonidet P-40 detergent (Sigma), and these proteins were then used as competitive inhibitors, as described above. Proteins from EF5-treated hypoxic cells were much more reactive than those at intermediate oxygen levels (1% oxygen). Proteins from EF5-treated aerobic cells or etanidazole-treated hypoxic cells were nonreactive (Fig. 5). The latter result confirms the specific requirement for EF5 and demonstrates that the reactivity described does not result from specific protein changes which could be associated with hypoxic growth conditions.

A final requirement was to determine whether the ELK2-4 antibody would recognize EF5-adducts in situ, using whole cells or thin sections, with or without fixation (Fig. 6, A-D). In the first example, 9L rat glioma cells were incubated for 4 h in air versus nitrogen in the presence of 0.5 mM EF5. Methods used were as described above to assess binding. The cells were trypsinized and allowed to attach to microscope slides; then they were air dried and stained overnight at 4°C with ELK2-4. Antibody binding was visualized using a fluorescein-labeled second antibody (The Jackson Laboratory) recognizing the isotype (mouse IgG1) of ELK2-4. No antibody binding was observed for the aerobic cells but the hypoxic cells fluoresced brightly (Fig. 6A). A second example illustrates the binding pattern observed after whole spheroids growing in aerobic medium in vitro were incubated for 4 h in the presence of 0.5 mM EF5 (Fig. 6B). It can be seen that the outer rim of the spheroid was completely unstained, but the staining intensity increases for cells in the spheroid interior which are expected to be hypoxic. There was no staining in the necrotic center of the spheroids, indicating the necessity of having viable cells to form adducts with the EF5 molecules.

The ability of the antibody to detect regions of high binding of EF5 given to tumor-bearing animals was also confirmed (Fig. 6, C and D). Tumors typically contain large amounts of host (mouse) antibody so that a second antibody could not be used to detect the ELK2-4 mouse monoclonal in the case of mouse tumors. To circumvent this problem, AMCA-conjugated EKL2-4 (Fig. 4) was used. High contrast areas were found both in EMT6 mouse and Morris hepatoma 7777 rat tumor tissue (Fig. 6, C and D, respectively).

**DISCUSSION**

The data presented in this report have demonstrated several qualitative aspects of EF5 metabolism which are now expected for 2-nitroimidazoles and other nitroaromatics, i.e., dramatic inhibition with increasing oxygen concentration (Fig. 2) and the ability to discriminate hypoxic from aerobic tissue (Fig. 6). In more quantitative terms, an oxygen-sensing drug should have the same characteristic dependence of its metabolism on oxygen concentration in all cells or tissues of interest. We have previously demonstrated conclusively that this is not the case for misonidazole (27, 29, 38, 39). Although the rate of bioreductive metabolism of misonidazole always has a large oxygen-dependent component, it is, in absolute terms, extremely variable from one cell/tissue type to the next, both in terms of absolute binding rate and in the oxygen dependence of the process. At this time, the cause of the variability in absolute binding rate for misonidazole is not clear. Possibly, there are enzymes (like DT-diaphorase) which can donate two electrons to misonidazole, thus bypassing the oxygen-dependent step. It is known that the DT-diaphorase activity of various cell types is highly variable (40). We have found that the nonoxygen-dependent variability associated with the metabolism of misonidazole is not shared with closely related compounds such as etanidazole (29, 38).

Etanidazole was developed with the principal objective of reducing patient neurotoxicity associated with misonidazole (41), but as a marker of hypoxia, it suffers from two disadvantages (29). First, most of the bioreductive metabolites of etanidazole are acid-soluble, presumably low-molecular-weight compounds which would be lost during typical histological processing. This property is thought to be caused by its extreme hydrophilicity (42). Secondly, etanidazole has no specific marker atom which could be used in noninvasive assays...
MoAb DETECTS HYPOXIC CELLS VIA BOUND 2-NITROIMIDAZOLE

Fig. 6. Immunohistochemical staining of cells and tissues by ELK2-4 after treatment with EF5. A (top left), compares the binding of antibody to 9L cells treated with 0.5 mM EF5 in the presence of either air (dark) versus nitrogen (light). The exposures of drugs, antibodies, and photography were all identical in the two halves of the figure. Only the oxygen concentration during incubation was varied. B (upper right), results of a similar experiment where whole spheroids (EMT6-Ro cells) growing in spinner flasks were incubated with 0.5 mM EF5 for 4 h. Ten-μm frozen sections are illustrated. A and B, slides were treated using ELK2-4 and a second fluorescein-containing antibody as described in “Materials and Methods.” C (lower left), another example showing 20-μm sections from an actual tumor-bearing animal. A BALB/c mouse bearing an EMT6 tumor received i.p. injections of 1.2 ml of 2 mM EF5. Sections were treated as above for the spheroid sections, except that only one antibody was used, namely the AMCA conjugated ELK2-4 described in “Materials and Methods.” The AMCA dye fluoresces in the blue, using UV excitation, but as above, the bright areas are those expected to contain hypoxic cells. D (lower right), results of a similar experiment using a buffalo rat bearing a Morris 7777 tumor.

such as PET, MRI-MRS, or single photon emission computed tomography. We initiated this work hoping that fluorine-containing derivatives of etanidazole would, like the parent drug, have limited nonoxygen-dependent variations in binding, while allowing the detection techniques offered by the fluorine atoms (PET; MRS-MRI). Additionally, the 5 fluorines of EF5 substantially decrease its hydrophilicity compared with etanidazole, thus causing a greatly decreased proportion of acid-soluble adducts. Although EF5 is much less polar than etanidazole, it maintains a reasonably high solubility in physiological saline at 37°C (~14 ms).

It was also reasoned that the pentafluorinated side chain of EF5 might be sufficiently nonphysiological as to allow the formation of highly specific antibodies. This may have been a contributing factor in the successful production of polyclonal antibodies to CCI-103F in the pioneering studies of Raleigh et al. (28, 43). This reasoning may have been justified, considering the current successful production of monoclonal antibodies to the present drug, EF5.

There are two aspects of the binding characteristics of MoAbs which are relevant to their experimental and/or clinical use, specificity and affinity. In determining the relative specificity of ELK2-4, a large number of antibodies from various hybridoma clones were examined. It was clear that the antibodies from most clones readily discriminated the antigenic adduct-containing proteins from untreated protein (data not shown); however, it appears that the considerable manipulations involved in protein disulfide reduction and adduct formation allowed the expression of many new protein epitopes, most of which had little to do with the actual EF5 drug adduct. We therefore realized that a very general screening mechanism was required and settled on whole-cell protein preparations prepared from either hypoxic cells (control) or hypoxic EF5-treated cells (experimental). This selection technique has allowed the choice of the ELK2-4 antibody which, to date, has been found to show no binding to non-EF5-treated tumor tissue. Much more work is presently required to test for this specificity in a broad variety of tissues.

The affinity of an antibody for its antigen determines the useful range of sensitivity and to some extent affects the optimization of its use (i.e., a low affinity antibody might typically show more rapid dissociation kinetics, and therefore its optimal use would require shorter times for rinsing, nonuse of secondary antibodies, etc.). The affinity of ELK2 (based on equilibrium dialysis experiments; data not shown) is of the order of 10⁶ for the parent drug and 5 to 10 times higher for protein adducts of EF5. Thus, the present antibody should be very usable at adduct concentrations of the order of 10 μM and higher. From the data in Fig. 2, it is clear that dramatically higher
levels can easily be achieved. For example, in hypoxic EMT6-Ro cells treated with 500 μM drug, the adduct level is more than 2 mM after only 2 h of incubation (i.e., 0.003 pmol/cell; cells have a size of ~1.5 μl). Previous studies with relatively nonpolar nitroimidazoles like misosidazole suggest that 500 μM drug levels would not be acceptable in humans. However, at drug levels which are likely to be completely nontoxic (e.g., 20 μM) EMT6-Ro cells still accumulate 15–300 μM drug over the range of oxygen levels typically expected from normal to extreme hypoxia (Fig. 2). Furthermore, it is quite likely that antibodies can be found with even higher affinities since only 10% of the levels can easily be achieved. For example, in hypoxic EMT6-Ro cells nontoxic in humans. However, at drug levels which are likely to be completely drug over the range of oxygen levels typically expected from normal tissue fixation techniques. Although we are in only the preliminary stages of characterizing this drug and have only tested two of the possible detection schemes, it appears that EP5 and ELK2-4 may allow the detection of hypoxia in vivo at high sensitivity and resolution.

REFERENCES


Detection of Hypoxic Cells by Monoclonal Antibody Recognizing 2-Nitroimidazole Adducts

Edith M. Lord, Lee Harwell and Cameron J. Koch


Updated version
Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/53/23/5721

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/53/23/5721. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.