The Hypoxia-Activated ProDrug AQ4N Penetrates Deeply in Tumor Tissues and Complements the Limited Distribution of Mitoxantrone

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Abstract

Hypoxic tumor cells are likely to be resistant to conventional chemotherapy, in large part because many anticancer drugs are unable to penetrate into poorly oxygenated tumor tissue. Here, we used quantitative immunofluorescence to study the distribution of mitoxantrone and AQ4N in tumor tissue. AQ4N is a prodrug activated under hypoxic conditions to AQ4, which is structurally similar to mitoxantrone and inhibits topoisomerase II. We characterized the penetration of mitoxantrone and AQ4N/AQ4 through multilayered cell cultures (MCC) and in relation to blood vessels and hypoxic regions in human tumor xenografts. We also studied tumor growth delay after treatment with each agent alone and with the combination. In both MCC and xenografts, mitoxantrone is taken up by proximal cells and penetrates slowly to distant regions. In contrast, AQ4N rapidly penetrates MCC and tumor tissue in vivo, and AQ4N (or its reduced form AQ4) is detected at high concentration within hypoxic regions. The targeting of mitoxantrone to oxygenated regions and AQ4N/AQ4 to hypoxic tumor regions results in effective drug exposure over the entire tumor after combined treatment and increases tumor growth delay compared with either drug alone. The combination of a clinically used anticancer drug with limited tissue penetration and a structurally related drug activated in regions of tumor hypoxia is an effective strategy to overcome chemoresistance due to the tumor microenvironment. This study supports clinical evaluation of AQ4N in combination with conventional anticancer agents, such as mitoxantrone.

[Research Article]

Introduction

Compared with normal tissues, blood vessels in tumors are often dilated and convoluted, and tumor blood flow is disorganized and variable. The imperfect vasculature of solid tumors leads to regions of reduced nutrient availability and hypoxia. Hypoxia in tumors may select for a more malignant phenotype (1) and has been shown to be an independent predictor of poor clinical outcome, most likely due to both drug and radiation resistance, and also to an increased risk of metastasis (2). Tumor regions distant from functional blood vessels may play a key role in resistance to chemotherapy for several reasons. Many drugs have limited penetration from tumor blood vessels to those distal regions (3, 4), and tumor cells within them tend to be slowly proliferating and consequently resistant to cycle active agents (5, 6). The stabilization of the heterodimeric transcription factor hypoxia inducible factor-1 during hypoxia allows tumor cells to adapt to their harsh microenvironment by stimulating the expression of a large number of hypoxia-related genes, including some which have been shown to reduce responsiveness to certain anticancer drugs (7).

It may be possible to exploit tumor hypoxia for cancer therapy through the use of hypoxia-targeted agents (8). Tirapazamine, the first hypoxia-activated prodrug to undergo clinical evaluation, has shown only modest clinical activity when used in combination with conventional chemotherapy (9, 10); one explanation for this modest clinical activity is the poor penetration of tirapazamine from tumor blood vessels into hypoxic regions (11, 12). To overcome the limitation of previous anticancer agents, it is essential to characterize not only their activity but also their relative distribution in the tumor microenvironment. Our group and others have developed models to quantify the penetration of anticancer drugs through tumor tissue in culture (multilayered cellular cultures, MCC) and in tumors of mice (3, 4, 11–15).

AQ4N (or banoxantrone; 1,4-bis(2-(dimethylamino)ethyl)amino)-5,8-dihydroxy-anthracene-9,10-dione bis-N-oxide) is a highly soluble di-N-oxide prodrug designed to have little cytotoxicity in the presence of oxygen but is selectively bioreduced in hypoxic environments to the cytotoxic alkylaminooanthraquinone metabolite AQ4 (16, 17). AQ4 is structurally related to mitoxantrone, intercalates DNA with high affinity, and inhibits topoisomerase II activity (17). In previous preclinical studies, AQ4N had minimal antitumor effect as a single agent but caused tumor growth delay when tumors were rendered hypoxic (18) or when used to treat tumors with large regions of hypoxia (19). AQ4N is being evaluated alone or in combination with conventional treatments in phase I and phase II clinical trials in patients with hematologic and solid tumor malignancies.

We hypothesize that the combination of mitoxantrone and AQ4N will result in effective drug exposure throughout solid tumors, leading to improvement in therapeutic index.

Materials and Methods

Drugs and reagents. AQ4N was supplied by Novacea, Inc., as a solution diluted in 0.9% sodium chloride (30 mg/mL, stored at 4°C). Mitoxantrone (Mayne Pharma) was obtained from the hospital pharmacy as a solution diluted in sodium chloride at a concentration of 2 mg/mL. Both drugs were injected i.v. into mice via the tail vein.

Purified rat anti-mouse CD31 monoclonal antibody was purchased from BD Pharmingen, and the Cy3-conjugated goat anti-rat IgG secondary antibody was purchased from Jackson Immuno Research Laboratories, Inc. The hypoxia-selective agent EF5 and Cy5-conjugated anti-EF5 antibody were provided by Dr. C.J. Koch (University of Pennsylvania).

Cell cultures and tumors. EMT-6 mouse mammary sarcoma cells were obtained originally from Dr. R. Sutherland (University of Rochester). The EMT6 cell line was selected because it readily allows the growth of MCCs, and we have previously studied the penetration of multiple drugs, including mitoxantrone, using this tissue culture model (13). MDA-MB-231 human
breast cancer cells, PC-3 human prostate cancer cells, and DU-145 human prostate cancer cells were purchased from American Type Culture Collection. We chose to study xenografts derived from human breast and prostate cancers because these types of tumor have moderate clinical sensitivity to mitoxantrone (20, 21). The PC-3 prostate tumor is well characterized, and we have already studied doxorubicin penetration in PC-3 xenografts (15). The MDA-MB-231 and DU-145 cell lines grow readily as xenografts in nude mice and are moderately sensitive to AQ4N/AQ4. The cell lines were maintained as monolayers at 37°C in a humidified atmosphere of 95% air plus 5% CO2 and 95% air–5% CO2, balance nitrogen, and stored at 70°C before tissue sectioning and immunohistochemical staining. Single cryostat sections (10-μm thick) were cut from each tumor. At least three different tumors were analyzed for each time/dose distribution experiment.

Drug penetration in vivo. In experiments to study drug penetration, mitoxantrone and AQ4N/AQ4 were given at doses of 40 mg/kg and 200 mg/kg, respectively, to facilitate detection and quantification of their autofluorescence. Animals received also 0.2 mL of 10 mmol/L EF5 i.p. 2 to 3 h before they were killed to identify hypoxic tumor regions (23). Animals were killed, and tumors were excised at 10 min, 24 h, and 48 h after drug injection. Tumors were embedded immediately in OCT compound, frozen in liquid nitrogen, and stored at −70°C before tissue sectioning and immunohistochemical staining. Single cryostat sections (10-μm thick) were cut from each tumor. At least three different tumors were analyzed for each time/dose distribution experiment.

Autofluorescence of AQ4N/AQ4 and mitoxantrone were detected using the Olympus BX50 fluorescence microscope, as described above, for MCC and using the Olympus UpPlanSapo 10×/0.40 objective lenses with identical signal amplification settings for each experiment. Tissue sections were tiled using an automated stage and dedicated software (Media Cybernetics In vivo). Using far-red filter sets, the background autofluorescence of the tumor tissue is negligible, and untreated tumors show a uniform low distribution of fluorescence intensity. Background fluorescence, representing the lowest fluorescence intensity within the tumor tissue in each experiment, is subtracted in all experiments. All images were converted to an identical fashion to those used to capture drug fluorescence. Animals received also 0.2 mL of 10 mmol/L EF5 i.p. 2 to 3 h before they were killed to identify hypoxic tumor regions (23).

Subsequently, tumor sections were washed in PBS and blocked with a protein-blocking reagent (ID Labs, Inc.) for 15 min to prevent nonspecific antibody binding. Sections were dualy stained with a rat anti-CD31 (1:100) and Cy 5-conjugated mouse anti-EF5 antibody cocktail for 1 h in a humidified chamber (antibody dilutions indicated in parentheses) and then stained with a Cy3-conjugated goat anti-rat IgG and Cy 5-conjugated mouse-anti-EF5 (1:50) antibody cocktail for 1 h in a humidified chamber. Some sections were dually stained with a rat anti-CD31, sections were stained with a rat anti-CD31, and then were stained with a Cy5-conjugated goat anti-rat IgG secondary (1:100) for 40 min to recognize rat anti-CD31. Sections were subsequently reimaged in an identical fashion to those used to capture drug fluorescence. Cy3 and Cy5 fluorescence were visualized using 530-nm to 560-nm excitation/573-nm to 647-nm emission filter sets and far-red filter sets, respectively. The expression of CD31 on endothelial cells allows recognition of tumor blood vessels, and CD31+ endothelial cells were pseudocolored in red. The uptake of EF5 identifies hypoxic regions (23), and those regions were pseudocolored in green. Composite images demonstrating drug fluorescence (blue), CD31+ blood vessels (red), and EF5+ hypoxic cells (green) were generated using Media Cybernetics Image Pro PLUS 6.0. Some sections were stained with H&E to identify viable cells and regions of necrosis.

The entire tumor cross-section was used to quantify the drug distribution in vivo; the tumor edge, areas of necrosis, and any artifacts due to processing were excluded. The method has been described in detail previously (15).

Figure 1. Influence of AQ4N on the surviving fraction of MDA-MB-231 human breast cancer cells, as evaluated by a clonogenic assay, under normoxic (solid line) and hypoxic (dashed line) conditions. Cell survival is presented as the ratio of colonies at a particular drug concentration to colonies in the untreated condition. Points, mean from three replicates; bars, SD (where bars are not shown, they are less than the height of the symbols).
to an 8-bit (255 arbitrary units) black and white binary image so that blood vessels were identified by an intensity of 255. The resultant image was overlaid with the corresponding 8-bit image of the tumor cross-section displaying drug fluorescence as a grayscale ranging from 0 to 254 units. Our computer-customized algorithm quantifies for each pixel the fluorescence intensity due to drug and the distance of pixels to the nearest blood vessel (recognized by the 255-unit fluorescence). These absolute values are integrated over the section to provide a plot of fluorescence intensity due to drug as a function of distance to the nearest blood vessel. The far-red filter sets gave minimal artifacts from background autofluorescence of the tumor tissue.

**Tumor growth and response to treatment.** When tumor xenografts grew to a size of ~5 to 8 mm in diameter, animals were sorted randomly and coded using ear tags into groups of five to six mice. The longest and perpendicular diameters of tumors were measured using calipers thrice weekly without knowledge of the treatment history of the mice. Tumor measurements were converted to tumor volume \((V)\) using the formula: \(V = W^2 \times Y / 2\), wherein \(W\) and \(Y\) are the smaller and larger perpendicular diameters, respectively. Body weight of mice was also measured thrice weekly. Mice bearing MDA-MB-231 and DU-145 tumors were treated with AQ4N alone, with mitoxantrone alone, with the combined treatment or with the vehicle solution.

**Results**

**Effects of AQ4N on clonogenic survival.** The colony-forming assay performed with both MDA-MB-231 (Fig. 1) and DU-145 human tumor cells (data not shown) treated with AQ4N showed that AQ4N has potent and selective cytotoxicity under hypoxic conditions. The IC\textsubscript{50} values for the MDA-MB-231 cell line exposed to AQ4N under aerobic and hypoxic conditions were, respectively, 3.7 and 0.06 \(\mu\)mol/L.

**Drug penetration through MCC.** Figure 2 shows the time-dependent penetration through MCC of mitoxantrone and AQ4N under aerobic conditions and AQ4N under hypoxic conditions. After 15 to 30 minutes, mitoxantrone accumulated mainly in the first cell layers. After 1 hour, drug fluorescence was seen on the Teflon membrane (Fig. 2A). In contrast, the produg AQ4N penetrated rapidly throughout the entire MCC under aerobic conditions and was observed on the Teflon membrane 5 minutes after application of the drug (Fig. 2B). Higher fluorescence intensity due to AQ4N/AQ4 (which have similar fluorescent properties and cannot be separated) was seen in the central (probably hypoxic) areas of MCC under aerobic conditions (Fig. 2B). AQ4N also penetrated MCC rapidly under hypoxic conditions, and fluorescence due to the drug (or its active metabolite) was more intense (Fig. 2C).

**Drug penetration in tumor xenografts.** The presence of large hypoxic regions (assessed by the EF5 staining) was seen in relatively small MDA-MB-231 xenografts (~40 mm\(^3\)) compared with PC-3 and DU-145 xenografts (>100 mm\(^3\)). Representative three-color composite images showing the distribution of mitoxantrone in relation to blood vessels and hypoxic regions in tissue sections from MDA-MB-231 (Fig. 3A), PC-3 (Fig. 3B), and DU-145 (Fig. 3C) xenografts are shown in Fig. 3. For clarity, error bars are excluded from this and subsequent figures, but they are almost nonoverlapping. Ten minutes after injection, distribution of mitoxantrone through each tumor xenograft was poor, with fluorescence due to drug localized in cells close to blood vessels; there was a rapid decrease in fluorescence intensity due to drug with increasing distance from blood vessels. H&E staining of tumor sections showed apparently viable cells far from blood vessels that were not exposed to detectable drug fluorescence. Forty-eight hours after injection, the fluorescence due to mitoxantrone was more uniform but at a low level in all tumor sections.
Similar, representative, three-color composite images show the distribution of AQ4N/AQ4 (Fig. 4A–C). Ten minutes after injection of AQ4N, each tumor xenograft showed homogeneous fluorescence intensity over the entire tumor sections. Graphic representation of AQ4N/AQ4 fluorescence distribution in relation to blood vessels confirms the deep penetration of the drug. At 48 hours, AQ4N/AQ4 concentrated in hypoxic regions far from blood vessels in both MDA-MB-231 and DU-145 xenografts whereas fluorescence due to drug was low in the regions around blood vessels (Fig. 4A and C). Accumulation of AQ4N/AQ4 in hypoxic regions was not observed within the PC-3 tumor sections (fluorescence close to the background level observed in control tumors; Fig. 4B).

As mitoxantrone and AQ4N/AQ4 have similar fluorescent properties, we could not distinguish them in tumor sections when both drugs were injected together. MDA-MB-231 and DU-145 tumor sections showed high and relatively uniform fluorescence intensity around blood vessels and far from blood vessels after combined treatment (Fig. 5A and B). Using identical signal amplification settings, we showed higher fluorescence intensity of the combined treatment compared with either drug alone in MDA-MB-231 tumor sections with substantial drug fluorescence in regions far from blood vessels (Fig. 5C). In both MDA-MB-231 and DU-145 tumors, fluorescence due to the combined treatment remains substantial over the entire tumor sections at 48 hours.

**Effects of combined treatment on growth of MDA-MB231 and DU-145 xenografts.** No substantial tumor growth delay was observed for any tumors after a single injection of 80 to 200 mg/kg AQ4N alone (as expected for an agent that is likely to be cytotoxic to hypoxic cells, which may make up only a small proportion of tumor cells). The response of MDA-MB-231 xenografts to combined treatment was compared with the response to either drug alone at the same dose (mitoxantrone 4 mg/kg, AQ4N 80 mg/kg; Fig. 6A). With a small increase in toxicity (maximum mean loss body weight of 15.6% compared with 10.8% in the mitoxantrone group), the combined treatment achieved greater tumor growth delay. Furthermore, similar growth delay of MDA-MB-231 tumors was observed after treatment with mitoxantrone at the maximum tolerated dose (7 mg/kg) as with combined treatment using low doses of mitoxantrone (3 mg/kg) and AQ4N (60 mg/kg; Fig. 6B). In this experiment, the toxicity of the combined treatment group was less [mean maximum loss of body weight of 13.6% (3.6–22.1%) compared with 24.1% (13.9–35.3%) in the mitoxantrone group]. For the DU-145 xenografts, identical low doses of the combined treatment (mitoxantrone, 3 mg/kg; AQ4N, 60 mg/kg) were used.
60 mg/kg) resulted in substantial tumor growth delay, but mitoxantrone alone at the maximum tolerated dose (7 mg/kg) achieved a greater tumor growth delay with similar loss of body weight (Fig. 6C).

Discussion

Poor distribution of some anticancer drugs to hypoxic and nutrient-deprived regions of tumors is an important and neglected cause of drug resistance (3, 4, 11–15, 24). Our hypothesis is that prodrugs, such as AQ4N, that are activated to potent cytotoxins under hypoxic conditions will improve the effectiveness of conventional chemotherapy by killing or inhibiting the repopulation of surviving hypoxic tumor cells, provided that they have the ability to localize in and kill hypoxic cells.

Our previous studies of the distribution of mitoxantrone in MCC (13) and the current study show that mitoxantrone has slow penetration through tumor tissue and relatively poor distribution in solid tumors. Mitoxantrone binds avidly to DNA and is a weak base, which may lead to sequestration of the drug in acidic endosomes within cells. This consumption of drug by proximal cells likely explains its slow penetration through tissue. Results showing fluorescence due to mitoxantrone at 48 hours after injection are consistent with delayed drug penetration due to the long terminal half-life of the drug in blood (25). The long half-life may allow diffusion or convection from blood vessels for prolonged periods, leading to a more uniform albeit low-level distribution; however, to kill cancer cells, drugs must be present in lethal concentration.

The response of tumor cells to AQ4 (the bioreduced and active form of AQ4N) varies with tumor cell type. Thus, AQ4N/AQ4 has limited toxicity under hypoxic/anoxic conditions against the PC-3 cell lines (data not shown; ref. 26). The mechanism of enzymatic reduction of AQ4N remains incompletely understood but seems to be mediated by multiple cytochrome P450 enzymes (27, 28). Because there is considerable variability in expression of cytochrome isoforms between tumor types, we hypothesize that low levels of a specific cytochrome isoform needed to activate AQ4N may impair efficient activation in certain experimental systems. Robson and colleagues showed that the antitumor efficacy of AQ4N could be increased by delivery of genes encoding the appropriate drug metabolizing cytochrome enzymes to syngeneic mouse tumors in a gene-directed prodrug therapy strategy (28, 29). The PC-3 cell line used in current experiments did not show hypoxia-selective sensitivity to AQ4N and AQ4N/AQ4 accumulation in hypoxic regions of PC-3 xenografts, perhaps due to failure to activate AQ4N to AQ4.

We were unable to distinguish AQ4N from its reduced form AQ4 in hypoxic regions of tumors. However, in vitro experiments with liquid chromatography and mass spectrometry in H460 lung adenocarcinoma cells treated with 8 μmol/L AQ4N for 12 hours under low oxygen conditions (0.2% O2, compared with 95% air–5% CO2)
showed selective accumulation of the bioreduced metabolite AQ4 and absence of AQ4N in the lysates of cells under hypoxic conditions (data not shown). Neither AQ4 nor AQ4N was detected in lysates of cells exposed to aerobic conditions. Furthermore, Atkinson and colleagues used matrix-assisted laser desorption/ionization mass spectrometry imaging to examine the distribution of AQ4N and AQ4 in tumor tissue. They found that, 24 hours after i.p. injection of AQ4N in tumor-bearing mice, the distribution of AQ4 is almost completely separate from that of AQ4N. Furthermore, the distribution of AQ4 overlapped with the distribution of ATP depletion, as is common in areas of hypoxia (30). Recently, in humans, AQ4 was shown using analytic and microscopic techniques to be tumor selective and to colocalize with the Glut-1 hypoxia marker in tumor biopsies after treatment in a phase I pharmacodynamic study of patients with advanced solid tumor malignancies (31). Although there is no pharmacodynamic study showing that the drug fluorescence in the hypoxic regions of our tumor models is due to AQ4, the observation that the drug fluorescence remains present for >72 hours (data not shown) is consistent with the persistence of highly stable AQ4 intercalation with DNA (26).

The xenografts evaluated in the present study seem to contain regions of diffusion limited hypoxia that occurs at distances of >100 μm from tumor blood vessels, although we also observed CD31+ cells in hypoxic areas that probably reflects nonfunctional vasculature (perfusion derived hypoxia). It has been shown previously that, in HCT-116 human colon cancer xenografts, cells located at the border of necrosis were the first cells to begin to proliferate after treatment with gemcitabine (24); this provides evidence that the intrinsic resistance of cells distal from tumor blood vessels is a cause of treatment failure. Furthermore, there is growing evidence that cancer cells with stem cell properties may be localized in the hypoxic regions of the tumor (32). As nutrition improves after lysis of cells closer to blood vessels, these poorly cycling hypoxic cells with stem cell properties may start to recycle and repopulate the tumor. Because AQ4 has avid and prolonged DNA-binding affinity, the repopulation of initially quiescent cells after improvement in their nutrition might be inhibited, and they might die as a result of toxicity of AQ4 when induced to proliferate. This hypothesis is supported by the results from our MDA-MB-231 xenograft model showing the low rate of tumor regrowth after combined treatment with AQ4N. In contrast, this result was not observed with the rapidly progressing DU-145 xenograft tumor, and future studies are needed to investigate repopulation of cells from hypoxic regions of different tumors after killing of proximal cells by conventional chemotherapy.

It is probable that use of a hypoxia-selective prodrug, such as AQ4N, will increase the therapeutic ratio for conventional chemotherapy when used to treat tumors with substantial hypoxia and which have the necessary enzymes for its activation.

Figure 5. Distribution of the combination of mitoxantrone and AQ4N/AQ4 in relation to blood vessels and regions of hypoxia in MDA-MB-231 (A) and DU-145 (B) xenograft sections 10 min and 48 h after i.v. injection. Blue, drugs; red, vessels; green, hypoxic regions. The graphs show, for each panel, the mean of absolute values of (background-subtracted) fluorescence intensity of the combined treatment for the different tumors, as a function of distance to the nearest tumor vessel, 10 min (solid blue line) and 48 h (dotted blue line) after the injection. Using identical signal amplification settings for MDA-MB-231 tumors, the graph (C) shows that the fluorescence due to combined treatment (red line) is higher compared with the fluorescence of either drug alone (mitoxantrone or AQ4N, blue lines) 10 min after injection. Scale bar, 100 μm.
Disclosure of Potential Conflicts of Interest

I.F. Tannock: Commercial research grant, Novacea, Inc.; A.S. Lalani is an employee of Novacea, Inc. (manufacturer of AQ4N). The other authors disclosed no potential conflicts of interest.

Acknowledgments

Received 2/22/2008; revised 8/26/2008; accepted 9/3/2008.

Grant support: Canadian Institutes of Health Research grant MOP-15388 (I.F. Tannock) and Novacea, Inc. Salary support to O. Trédan was provided by the Bourse Fondation de France.

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We thank the staffs of Advanced Optical Microscopy Facility at University Health Network and of Pathology Research Program of University Health Network for their assistance and J. Curd, K. Patel, R. Grantab, A. Fung, C. Lee, I. Wu, J. Caloiguan, and K. Laderoute for consultation and/or technical assistance.

References


Figure 6. Tumor volume and body weight as a function of time after treatment for mice bearing xenografts derived from MDA-MB-231 (A and B) and DU-145 (C) cells. Mice bearing MDA-MB-231 xenografts (A) were treated on day 0 with a single injection of mitoxantrone (4 mg/kg; blue curve), AQ4N (80 mg/kg; green curve), or the combination of both drugs (red curve). In the other panels, mice bearing MDA-MB-231 xenografts (B) or DU-145 xenografts (C) were treated on day 0 with a single injection of mitoxantrone at the maximum tolerated dose (7 mg/kg, blue curves), AQ4N (200 mg/kg, green curves), or the combination of both drugs with reduced doses (3 mg/kg mitoxantrone and 60 mg/kg AQ4N, red curves). Control mice received vehicle solution (black curves). Points, means for at least eight tumors or four mice; bars, SE.
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