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# Unusual Oxygen Concentration Dependence of Toxicity of SR-4233, a Hypoxic Cell Toxin[1]

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## ABSTRACT

**Toxicity from drugs activated by bioreductive metabolism has been suggested as a means to eliminate the treatment resistance caused by hypoxic tumor cells. In general, drugs have been selected to maximize the hypoxic cytotoxicity ratio [exposure (drug concentration × time) in air:exposure in nitrogen] to cause equal toxicity. On this basis, two recently developed drugs have very similar characteristics; an aziridine derivative of misonidazole (RSU1069) and a benzotriazine di-*N*-oxide (SR4233). The oxygen dependence of the toxic response has not previously been characterized. This report shows that the toxicity from SR4233 extends over a much greater range of oxygen concentrations than does that of RSU1069. Furthermore, unlike all previous drugs studied, the toxicity of SR4233 does not level off at high oxygen concentrations, but continues to decrease as the oxygen concentration increases. For 1 mM oxygen (the solubility of oxygen in medium at 37°C equilibrated with 100% oxygen and water vapor) the toxicity from SR4233 is at least 2000-fold less than that for hypoxia. Modeling the effect of oxygen on combined radiation and toxicity shows that radiation plus SR4233 should be much more effective in eliminating hypoxic cells than radiation plus RSU1069. The unusual oxygen dependence of toxicity by SR4233 may indicate a unique biochemical activation process.**

## INTRODUCTION

An important goal in cancer treatment is the identification and elimination of treatment-resistant cells. Because of their rapid growth, cancerous cells can become relatively isolated from the blood supply, and it becomes increasingly difficult for nutrients, especially oxygen, to diffuse to them, resulting in hypoxia. Hypoxic cells are very resistant to radiation damage, and the same diffusional limitations for oxygen can apply to the reactive, cytotoxic drugs used in chemotherapy. Thus, hypoxic cells are often used as a general model for treatment resistance (1, 2). Hypoxic cells in human tumors have been measured directly by using oxygen microelectrodes (3–7) and have been inferred in numerous other ways (8, 9). Various demonstrations of superior response to radiation with some human tumor types by using hyperbaric oxygen or nitroimidazoles continue to support the importance of hypoxic cells in determining the treatment response of primary tumors (10, 11).

Suggested treatment strategies involve selective targeting of the oxygen-deficient cells, either by augmenting other forms of therapy, for example by using hypoxic-cell-radiosensitizing agents (12), or by using direct-acting drugs which are specifically cytotoxic to the radiation-resistant cells (13). Two classes of drug have been shown previously to be selectively toxic to hypoxic cells (Fig. 1), the mechanism being related to their activation through bioreductive pathways (14, 15). These include nitroaromatics and quinone antibiotics. However, a problem with the effective use of these drugs lies in the oxygen dependence of the toxic process; cells become increasingly radiation resistant below about 30 torr (mm of Hg) oxygen partial pressure (16, 17), whereas the drugs mentioned above have their greatest increase in cytotoxicity at much lower oxygen levels; below about 3. torr for the nitroimidazoles (18–21) and 0.3 torr for mitomycin C (22). In other words, the oxygen concentration dependence of the cytotoxic and radiosensitivity processes need to complement each other, but they do not (Fig. 1).

Of equal or greater importance to the oxygen concentration dependence of bioreductive cytotoxicity, is the relative toxicity of the drug under "hypoxic" *versus* "aerobic" conditions. The HCR[3] has been defined as the ratio of drug exposures (concentration × time) for equal toxicity of aerobic *versus* hypoxic cells (23). For example, mitomycin C has a HCR of approximately two (see Fig. 1). This drug is also an extreme example of the problem indicated above since inhibition of toxicity occurs at submicromolar concentrations of oxygen (22).[4] This means that the maximal effect of oxygen inhibition of cytotoxicity occurs at oxygen levels which are so low that drug-resistant cells remain fully radiation resistant over an intermediate oxygen range. Furthermore, such cells would have a survival advantage because of their low but non-zero oxygen metabolism [previous studies have shown that the toxicity caused by extreme hypoxia alone can be prevented by as little as 0.15 torr oxygen (24)].

The nitroheterocyclic drugs ($RNO_2$), known as hypoxic cell radiation-sensitizing agents or radiosensitizers, are the best characterized example of hypoxia-specific toxic drugs. They can reverse the radiation resistance of hypoxic cells (1, 12, 25) and, through metabolism-linked processes, augment other forms of therapy. Consequences of this metabolism include depletion of cellular thiols (26), cytotoxicity (13, 27–29), enhanced response to chemotherapeutic agents (20, 30), enhanced radiosensitization (31, 32), enhanced response to phototherapy (33), decreased repair of radiation damage (34), production of toxic oxygen derivatives (15), and adduct formation to cellular (macro) molecules (25, 35, 36). Misonidazole, a 2-nitroimidazole, has been studied exhaustively in tissue culture, animals, and humans for its potential to act as either a radiosensitizer or cytotoxin specific for hypoxic cells. The HCR is about 20 or so for this compound (21, 37). All of the metabolic and toxic effects of misonidazole and other nitroaromatics are much more potent at low oxygen concentrations, and it would appear that these drugs have all the properties necessary to eliminate hypoxic cells. Unfortunately, another problem affects the clinical potential of drugs like misonidazole; they have been too toxic to use in human patients at the concentrations necessary to exploit fully, indeed even partially, the majority of the above phenomena (38, 39).

Recently two new classes of bioreductive drug have been developed. The prototype (RSU1069) for the first class, aziridine derivatives of nitroimidazoles, is toxic at dramatically lower concentrations than the parent drug (for extremely hypoxic V79 Chinese hamster cells, 5 μM RSU1069 *versus* 5 mM misonidazole) and has been shown to have a HCR of 30 to 200, significantly greater than any previous nitroimidazole (40). A completely different drug structure, benzotriazine di-*N*-oxide, accounts for the other drug class, typified by the compound SR4233. This drug is toxic in the same concentration range (5 μM for extreme hypoxia) and is reported also to have an HCR of

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[3] The abbreviation used is; HCR, hypoxic cytotoxicity ratio.

[4] C. J. Koch, unpublished observations.



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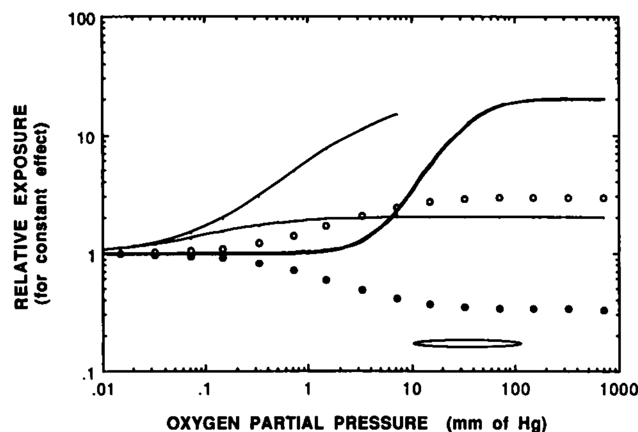


Fig. 1. Relative exposures for constant effect, computer generated by using parameters derived from the literature. The bottom curve in the figure (●) traces the well-known dependence of radiation exposure on oxygen partial pressure, characterized by a $C_2$ of ~2, $C_1$ (normalized to 1.0 for all curves) and $K$ of ~3 torr (17); see "Appendix" for a description of the parametric constants. A drug whose exposure characteristic is the mirror image of that for radiation (○) might still be inadequate, considering that the drug exposure is systemic; thus, drug exposure must be limited to the maximally sensitive normal tissue. An example of this is illustrated by the 2-nitroimidazole, misonidazole (——). It has a very low $K$, but a very substantial maximum difference between the toxic exposure in air *versus* nitrogen (21). Unfortunately, this drug is only cytotoxic to tumor cells in the millimolar and higher range, whereas toxicity, due to peripheral neuropathy, limits its use in patients to about 100 μM (curve does not extend beyond 10 torr because of drug solubility limitations of 100 mM). Toxicity from mitomycin C (—●—), a quinone antibiotic, is characterized by an extremely low $K$, and differential toxicity of only ~2 (22). Thus, an "ideal" drug (——) might require a much larger hypoxic:aerobic cytotoxicity than that shown for radiation, and the oxygen dependence might need to be right shifted, to provide more toxicity to the tumor cells while still sparing normal tissue (the $pO_2$ of venous blood is ~35 torr).

50–200) (23). A puzzling aspect of studies with these two drugs has been that, in different laboratories, each drug has been identified as having the highest HCR. Recently, Brown and Lemmon (37) have shown that the HCR varies substantially for both drugs with different cell lines; in particular that the HCR is much less for human than for rodent cell lines. We believe that an additional partial explanation for this discrepancy lies in the unusual dependence on oxygen of the toxicity of SR4233, and report herein that the toxicity of this drug continually decreases with increasing oxygen concentration. There is no evidence for saturation of this decrease as the oxygen levels are increased above air (20.9% oxygen) to 100% oxygen. Furthermore, the toxic effects of SR4233 are relatively constant throughout the entire range of oxygen concentrations allowing resistance to radiation (0–50 μM or 0–36 torr). Both of these characteristics are completely unlike all previous bioreductive toxic drugs studied. Modeling the combined toxic effect of radiation plus SR4233 (assuming additive responses) indicates substantially more efficient killing than other radiation-drug combinations over a broad range of oxygen concentrations.

## MATERIALS AND METHODS

The methods for cell culture and assay of cytotoxicity have been described (41). Since the control of oxygen concentration (22, 42) is crucial to the present experiments a brief description of these methods follows. Cells (WNRE subline of V79 Chinese hamster fibroblasts, originally provided by Dr. J. D. Chapman, Fox Chase Cancer Center, Philadelphia, PA) were inoculated onto the central area of glass Petri dishes and incubated overnight for firm attachment. On the morning of an experiment, drugs (where required) were added to minimal essential medium (Earle's salt base with 20% of normal bicarbonate (~5 mM) and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). All subsequent operations, before incubation of the cells for the cytotoxic exposure, were performed at temperatures near 4°C.

The existing medium on the dishes was replaced by the experimental medium, first as a rinse (1 ml) which was aspirated, and then as the final medium for the experiment (1 ml). The dishes were placed in leak-proof aluminum chambers (43). The lids of the chambers were held in place by symmetrically spaced bolts with an interior Viton O-ring seal. The chambers had a single gas connection port, which could be opened or closed via a bellows valve (Nupro, 4BK) and leak-proof connecting fittings (Cajon, VCO). The chambers were connected to a vacuum/gas manifold and the air in the chambers was replaced with gas containing the desired oxygen content via a series of gas exchanges (42). Since the cells were confined to the central area of the dish and only 1 ml of medium was used (resulting in a uniform liquid layer of approximately 0.1 mm thickness over the cells), the time constant of gas equilibration between the gas and liquid phases was only 6 seconds (42). However, the overall time for achieving gas equilibrium everywhere in the chambers immediately after a gas exchange, as measured by a polarographic oxygen sensor (44) sealed into the lid of the chamber was substantially longer (caused by the slow diffusion and convection within the complex multiple spaces inside the dishes and chamber). Therefore, the gas exchange procedure was typically 30 min in duration (42) and the chambers were kept cold during this interval by using crushed ice.

At the end of the gas exchange interval, the valves were sealed. Then the chambers were disconnected from the manifold and were warmed quickly to 37°C by immersion in a water bath, dried, and transferred to a warm room 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) (17).

After the incubation period, the chambers were opened and the dishes were immediately cooled on ice-cold aluminum trays. The drug-containing medium was removed and the dishes were rinsed twice with Earle's balanced salt solution followed by the addition of 0.05% trypsin (200:1 dilution of Sigma 40×) in calcium- and magnesium-free Earle's balanced salt solution. After cell detachment, serum-containing medium was added to stop the trypsin action and a portion of the cell suspension was counted. Various numbers of cells were plated into medium-containing plastic tissue culture dishes (Corning; 100 mm) and colony formation was assessed after 8–9 days' incubation. The numbers of cells added were adjusted to give approximately 100–300 surviving cell colonies (defined as containing >49 cells) per plate.

In past experiments, there was virtually no toxicity from compounds in air, so use of cold temperatures before and after incubation was much less important. In the present experiments, particularly with SR4233 at very high drug and oxygen concentrations (*e.g.*, >10 mM drug, 100% oxygen), exposure to air at the end of the drug exposure represented a substantial decrease in oxygen partial pressure and hence a more toxic condition than that of the gas phase during cytotoxic exposure, so it was important to reduce any toxicity during the medium change and gas exchanges by using temperatures near 4°C.

We have found that toxicity varies in a nearly linear fashion with "exposure" (*i.e.*, drug concentration multiplied by hours at 37°C, although experiments were seldom carried out for exposure times >6 h) (21, 37). Thus, drug effects were compared by estimating the drug exposure to produce a surviving fraction of 0.01. However, in all cases, several drug concentrations were used at each oxygen concentration in order to produce complete survival curves over a 2–6-hour period. Other researchers have found somewhat more complex relationships between exposure and killing (31), and these discrepancies remain unresolved at present, but they may relate to the very large number of factors which affect cytotoxicity, particularly by misonidazole (41).

A simple model for oxygen-based inhibition of toxicity, and additive interactions of radiation and drug toxicity was used to compare various drug-radiation interactions (see "Appendix").

## RESULTS

As indicated above, cytotoxicity was found to be linearly related to drug exposure for both RSU1069 and SR4233 (Fig. 2). For WNRE cells the HCR, defined above as the ratio of exposures for equal toxicity in air *versus* extreme hypoxia, was about 100 for both drugs. However, the oxygen concentration dependence of cytotoxicity was dramatically different for the two compounds (Fig. 3). RSU1069 showed the most significant change in toxicity over an oxygen concentration range of 2 to 20 torr, with only an additional 2- to 3-fold additional change between 20 and 725 torr oxygen (see also Ref. 45 for representative data for the 9L rat glioma cell line). In contrast,



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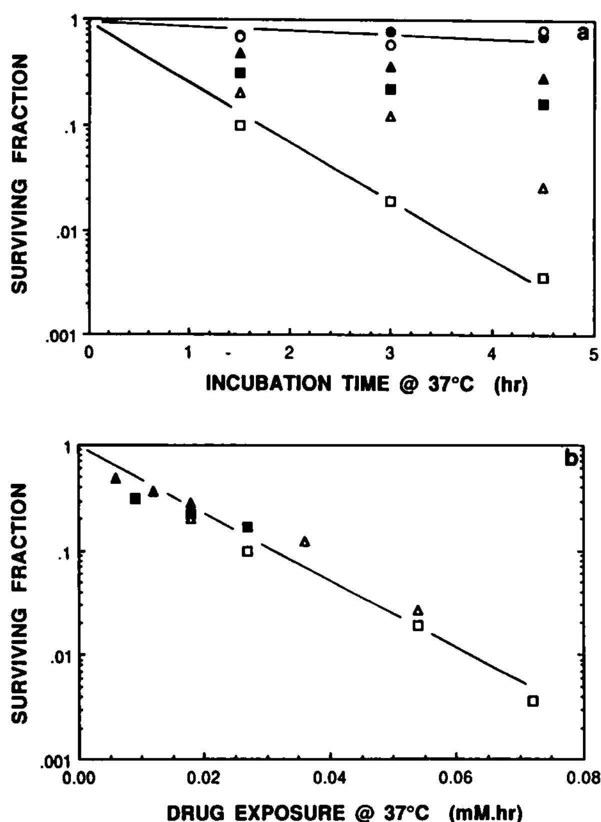


Fig. 2. Cell killing by SR4233. The data in *a* were from an early experiment where, based on results with other bioreductive drugs, we had anticipated substantial differences in toxicity between extreme hypoxia (less than 0.005 torr oxygen) and 0.08 torr oxygen. The symbols are for non-drug controls (○, ●), and 4, 6, 12, and 18 $\mu$M SR4233 (▲, ■, △, □). *Closed symbols*, extreme hypoxia; *open symbols*, 0.08 torr oxygen. *Straight lines* are drawn through only the plating efficiency and highest exposure to avoid confusion. *b* illustrates the drug-treated toxicity data, where individual points at a given concentration and time have been converted to exposure (drug concentration × time). Such data summaries then provide a single estimate of exposure for a given effect at each oxygen partial pressure. These data show that exposure provides an excellent measure of toxicity, and that, for this drug, there is no significant difference between the two oxygen levels.

toxicity from SR4233 was only moderately sensitive to oxygen from the lowest values measurable (0.2 torr) to 20 torr, but then increased sharply with an upward concave shape which appears to be still increasing even at 725 torr oxygen [exposure of this cell line to 725 torr (100% oxygen) was not itself toxic over the 6-hour drug exposure period used]. We have only limited data for the quinone antibiotic mitomycin C, but its dependence of toxic exposure on oxygen concentration is similar to that reported by Marshall, *e.g.*, maximal inhibition of toxicity at ~1 torr oxygen (22).

## DISCUSSION

The use of a ratio such as HCR is clearly inadequate for drugs like SR4233 whose oxygen dependence of cytotoxicity is most rapidly changing at aerobic levels of oxygen and above. The oxygen concentration dependence of cytotoxicity for SR4233 may explain an interesting anomaly found in the literature regarding this drug. Substantial differences have been reported in the HCR for SR4233 by different investigators, even for similar cell lines (23, 46). A possible cause for these differences is the failure to control adequately the oxygen level under aerobic conditions. The use of aerobic to describe culture conditions usually is meant to imply that the gas phase of the culture vessel contains air (plus water vapor, often with ~5% carbon dioxide). However, the formation of oxygen gradients due to cellular respiration is well documented in the literature, and these gradients vary tremendously with geometrical factors, cell density, and respiration rate (47–50). Since the toxicity of previous classes of hypoxic cell cytotoxins is nearly independent of oxygen concentration at aerobic levels, these gradients of oxygen would have been expected to have no effect on cytotoxicity. For example, we found that the cytotoxicity by RSU1069 to 9L cells varied only slightly between 72.5 and 725 torr oxygen (45). However, it can be seen from the data in Fig. 3 that the cytotoxicity of SR-4233 varies most strongly in precisely this high range of oxygen partial pressure. Therefore, it is essential to maintain cells in a well-stirred or shaken state (*e.g.*, this study) to determine accurately the toxic effects of drugs like SR4233. Our results may also provide an explanation for the excellent cytotoxicity of SR4233 against hypoxic cells *in vivo* (37), whereas other bioreductively activated drugs have required additional interventions such as clamping of the tumor vasculature (46). The reduction in blood flow imposed by clamping causes the oxygen partial pressure to decrease to the near-zero values required for effective cytotoxicity by other cytotoxic drugs.

It is interesting that the tendency of oxygen to react with free radicals is responsible for the opposing actions of oxygen in both radiation and bioreductive drug toxicity. When electron-affinic drugs are reduced in one-electron steps, free radicals are formed which have

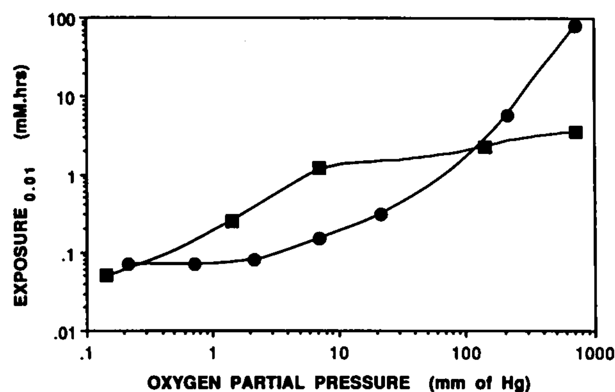


Fig. 3. Data such as are shown in Fig. 2*b* were summarized to find the exposure for 1% survival as a function of the oxygen partial pressure during the incubation period for SR4233 (●) and RSU1069 (■). *Points*, summary of data from several survival curves as illustrated by Fig. 2. In contrast to the curves for the drugs described in Fig. 1, cytotoxic exposure by SR4233 is nearly constant over the whole range of resistance to radiation damage, but increases rapidly with oxygen as the oxygen partial pressure is further increased. The required exposure has increased 20-fold at venous $pO_2$, more than 100-fold at aerobic levels of $pO_2$, and more than 1000-fold in an atmosphere of pure oxygen. There is no suggestion of a plateau throughout this range.

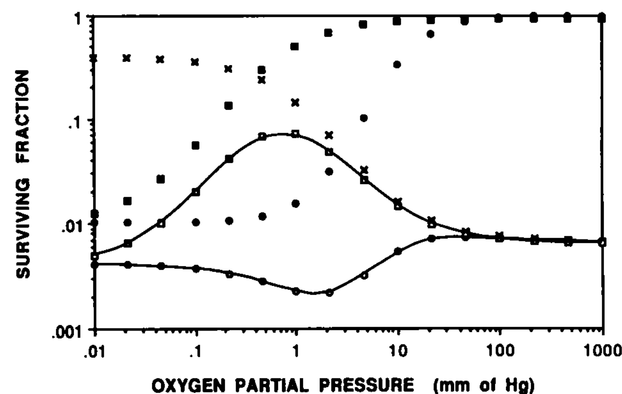


Fig. 4. Calculated oxygen dependence of the cytotoxic effects caused by a constant exposure to radiation (x), SR4233 (●), or RSU1069 (■). The exposures were chosen to give a surviving fraction of 0.01 (relative to controls) at the oxygen concentration of maximal sensitivity. Combinations of radiation with either SR4233 (○) or RSU1069 (□) were assumed to result in "additive" toxicities (so the relative surviving fractions were simply multiplied together at each oxygen concentration to give the combined surviving fraction).



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an extra electron. These radicals can undergo electron transfer to oxygen, leading to unchanged parent drug and superoxide [the superoxide is inactivated by cellular enzyme defense mechanisms, including superoxide dismutase and catalase (15)]. It is typically found that inhibition by oxygen of toxicity from reductive metabolism of drugs occurs at extremely low oxygen concentrations (19, 21, 41). The enhancing effect of oxygen on radiation sensitivity, which occurs at somewhat higher oxygen concentrations (16, 17), is also thought to be a free radical-related process whereby oxygen is thought to add to carbon radicals on cellular targets such as DNA (51). The resulting peroxy radicals then undergo further reactions which are poorly characterized.

The differences in oxygen dependency of the two processes may thus relate to the differences in radical chemistry: for radiation sensitization to occur, the oxygen molecule must add to target radicals, but for the reversal of bioreductive drug toxicity, it is only necessary for an electron to be transferred from the drug radical to oxygen (14). Thus, cells which have enough oxygen to survive and grow (5 to 50 torr range), remain relatively radiation resistant but are almost immune to the cytotoxic effects of bioreductive drug metabolism.

In the past, efforts to increase the radiation response of hypoxic cells in tumors by, for example, having the host breathe pure oxygen, or a mixture of 95% oxygen and 5% carbon dioxide (carbogen), have sometimes failed because greater effects were found in the normal than in the tumor tissue (the "steal" effect) (11, 52–54). On the one hand, the problem of the steal effect could possibly be turned to an advantage by the use of a drug like SR4233, since its toxicity would remain constant for the tumor (no change in $pO_2$) but be decreased for the normal tissue (increased $pO_2$) in such situations. On the other hand, in cases where both the tumor and normal tissue were reoxygenated by breathing hyperoxic gas mixtures, advantage could also be gained by the use of SR4233 with astute timing of drug delivery. The drug could be given before radiation therapy, thereby decreasing the hypoxic cell fraction; when the patient subsequently breathed high oxygen, tumor cell radiosensitization as well as reduced normal tissue drug cytotoxicity would occur. One could also imagine the use of hyperoxia as a means of reducing the effective half-life of the drug (*e.g.*, to reduce toxicity while administration of hyperoxia was made).

Even though the oxygen dependence of radiation sensitivity was not complementary to any of the drugs tested, all (with the exception of mitomycin C) show much larger ratios of toxicity to hypoxic relative to aerobic cells than does radiation (hypoxic cells are about 3-fold more resistant than aerobic cells; in other words, a 3-fold higher radiation dose is required to kill the same fraction of hypoxic cells compared with aerobic cells). Thus, an obvious question arises: why should not all of the illustrated drugs (with the exception of mitomycin C) be extremely efficient at eliminating hypoxic cells? One answer may lie in the method of agent administration and relative sensitivity of normal tissue at risk from the treatment.

With the exception of whole-body radiation for disseminated disease like Hodgkins' lymphoma, radiation therapy is delivered locally to the tumor, to the maximum tolerated dose for the normal tissue(s) involved. Furthermore, radiation dosage to normal tissue is minimized by irradiating the tumor from several different directions, in each case including the tumor volume but different normal tissue volumes. In marked contrast, chemotherapy agents expose the entire organism, and the dose of cytotoxin must be limited by the most sensitive normal tissue involved in the entire host. Thus for drugs like misonidazole, it can be shown that to achieve 1 log of cell killing (within a time frame compatible with the drug half-life) requires of the order of several mM concentrations of drug, even under conditions of maximal sensitivity (extreme hypoxia). However, the maximum drug dose tolerated by humans limits the concentration to approximately 100 $\mu$M.

Thus, the cytotoxic properties of chemotherapy drugs may need to have extremely high HCRs to be clinically useful, and furthermore, the oxygen concentration dependence of toxicity may have to be tailored for a specific application. The data presented here indicate that this latter property is highly variable with different drugs. This presumably relates to the precise mode of biochemical activation of the drugs involved, and/or the oxygen reactivity of the toxic species, metabolites, or radical intermediates. One might imagine that such tailoring will allow drugs to be developed for a specific range of oxygen concentrations.

An example of this aspect of drug/radiation interaction is illustrated in Fig. 4, where the combined effects of radiation and cytotoxicity are calculated as a function of oxygen concentration (based on data from this report and the literature; see "Appendix" for mathematical models). Using a dose of radiation which would kill 99% of aerobic cells, or a dose of drug which would kill 99% of hypoxic cells, the combined effect of both agents was calculated as a function of oxygen concentration (assuming additivity of agent interaction; see "Appendix" for model). It can be seen that the combination of radiation with RSU1069 leads to a substantial increase in survival at intermediate oxygen levels. This is particularly problematic, since moderately hypoxic cells are not under severe metabolic stress. In dramatic contrast, the combination of radiation with SR4233 actually causes a substantial decrease in survival at intermediate oxygen levels, with all cells at lower than normal oxygen levels having reduced survival.

In summary, we have shown that the oxygen concentration dependence of toxicity by SR-4233 is unique; exposure for a given level of toxicity has a plateau throughout the low $pO_2$ range typified by radiation resistance, and then continuously increases as the oxygen concentration increases. At 1 atmosphere of oxygen, the drug exposure required for a given level of toxicity is more than 2000 times that for hypoxic cells. The present results have important implications for the use of "bioreductive drugs" in the elimination of therapy-resistant hypoxic tumor cells. Now that a completely different type of oxygen-dependent toxicity has been found, it may be possible to tailor-make a drug or drug combination to be toxic over a specific oxygen concentration range. Studies on the mechanism of toxicity which make this compound so unique should prove helpful to our general understanding of toxic mechanisms for reductively activated drugs, which include many mutagenic and carcinogenic compounds.

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## APPENDIX

Damage by radiation or bioreductive cytotoxic agents is often considered to include two components (55), the first ($C_1$) oxygen independent, and the second ($C_2$) oxygen dependent. Oxygen-dependent damage is normally mediated by oxygen's high rate of reaction with chemical radicals (molecules with an unpaired electron). In the case of radiation damage, radicals formed on DNA are oxidized by oxygen, leading to increased damage with increasing oxygen, *e.g.*,

$$\text{Damage}_{\text{X-ray}} \sim C_1 + C_2\left(\frac{[O_2]}{[O_2] + K}\right) \quad \text{(A)}$$

In the case of bioreductive drugs, drug radical anions are formed which require further chemical reduction to the toxic species [in the case of nitroheterocyclics for example, to highly chemically reactive nitroso or hydroxylamine derivatives (56)]. Interaction of oxygen with these radicals leads to decreased damage



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since, by scavenging an electron from the drug radical, oxygen can prevent overall drug reduction, *e.g.*,

$$\text{Damage}_{\text{drug}} \sim C_1 + C_2\left(1 - \frac{[O_2]}{[O_2] + K}\right) \tag{B}$$

In both cases, $K$ has the same meaning as with the $K_m$ for enzyme kinetics, that is the concentration (of oxygen) for half-maximal effect. Exposures for constant effect are simply the inverse of the above equations describing damage. In other words, if damage is reduced by a factor of 5, then a 5-fold increase in exposure is required. For constant effect:

$$\text{Exposure}_{\text{X-ray}} \sim \frac{[O_2] + K}{[O_2](C_1 + C_2) + C_1K} \tag{C}$$

$$\text{Exposure}_{\text{drug}} \sim \frac{[O_2]C_1 + (C_1 + C_2)K}{[O_2] + K} \tag{D}$$

To calculate survival at a constant exposure, the form of the dose:survival-response function is required. For radiation response, the two parameter "linear-quadratic" equation was used;

$$\text{SF}_{\text{X-ray}} = e^{-\alpha D - \beta D^2} \tag{E}$$

where SF is the surviving fraction (normalized to unirradiated control) and $D$ is the radiation exposure. As mentioned above, the surviving fraction of drug-treated cells was well fitted by a simple exponential. Therefore the following function was used;

$$\text{SF}_{\text{drug}} = e^{-\gamma E} \tag{F}$$

where $E$ is the drug exposure in mM·h. Finally, the combined survival due to radiation plus drug was just:

$$\text{SF}_{\text{comb}} = \text{SF}_{\text{X-ray}} \times \text{SF}_{\text{drug}} \tag{G}$$

(*i.e.*, simple additivity of effects was assumed).

Although there may be many implications for the actual values of the constants used in the above equations, their evaluation and error analysis were not particularly relevant for the task at hand, which was simply to compute the best fit of oxygen dependence of constant effect for the various treatments. A simple algorithm was used which calculated the minimum residual sum of squares for experimental *versus* model data (57). The simple competition model described above for oxygen inhibition of toxicity provided an excellent fit to the RSU1069 data, but a very poor fit to the data for SR4233 (data not shown). Since, as mentioned in the previous paragraph, all that was necessary for the present calculations was to find a suitable mathematical fit to the data, a simple polynomial function of oxygen concentration was then tried for the SR4233 data. The fit to the data points was excellent, so this model was then used for the calculations of survival *versus* oxygen concentration of combined drug plus radiation (see Fig. 4). The basis for the unusual kinetic dependence of SR4233 toxicity on oxygen concentration is under present investigation.

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