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COMMENTARY

HYPOXIA AND DRUG METABOLISM

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Hypoxia is a subnormal oxygen concentration in cells that causes altered biochemical, physiological, or pathological function. It is a very common and serious aspect of many disease processes; over half of the deaths occurring in this country are listed as being of cardiovascular or respiratory origin [1], and most of these involve some degree of hypoxia. Hypoxia is an important consideration in pharmacology because (a) altered cellular function may alter the therapeutic effectiveness of an agent, (b) therapeutic agents may potentiate or protect against hypoxic pathology, (c) hypoxia may increase or decrease drug-induced toxicity, and (d) hypoxia may alter the rate of drug metabolism and, thus, the effective therapeutic dose.

The molecular events responsible for the altered hypoxic functions are deficient metabolic activities of enzymes that require molecular oxygen as substrate, i.e. the oxidases and oxygenases. These enzymes are widespread in nature and have important functions in drug metabolism. Since these enzymes have different affinities for oxygen, which is frequently expressed as the apparent $K_{m(O_2)}$ values, it follows that different functions have different sensitivities to the severity of hypoxia. Unfortunately, there is no simple way to satisfactorily measure and express the severity of hypoxia on a cellular basis *in vivo*. Definition of oxygen concentration is complicated by the difficulty of obtaining reliable measurements, by the existence of oxygen concentration gradients in cells and tissues, by the heterogeneity of cell types and organization of vascularization, by time-dependent variations in perfusion, and by the rapidity with which oxygen is consumed relative to the oxygen concentrations available. Oxygen concentrations in tissues *in situ*, measured by micropolarography, range from less than 1 to over 100 μM [2]. During hypoxia, the distribution is shifted to lower oxygen concentrations. Because there is no practical means to assess this clinically, hypoxia is expressed in terms of the arterial P_{O_2} as values below the normal range of 75 to 100 Torr. [3].

Oxygen is required for drug-metabolizing systems directly as a substrate for drug oxidations, as a terminal electron acceptor that controls other processes dependent upon the cellular redox state, and as the terminal electron acceptor in the mitochondrial synthesis of high-energy bonds required for drug transport and conjugation reactions. Since the oxygen dependencies of various oxidases and oxygenases have been found not to be coupled to the oxygen dependence of cytochrome oxidase, it is possible to distinguish between the consequences of hypoxia that are due to these other oxygen-dependent enzymes and those that are due to cytochrome oxidase [4]. *Bioenergetic hypoxia* occurs when functional changes are due to $[O_2]$ values that are insufficient to maintain normal cytochrome oxidase function, i.e. maintenance of the high-energy phosphate supply and proper oxidation–reduction state of pyridine nucleotides. *Metabolic hypoxias* occur when functional changes are due to $[O_2]$ values that are insufficient to maintain normal activities of other oxidases and oxygenases. Systematic studies of the effects of hypoxia on drug-metabolizing systems are not available, but current evidence suggests that, in general, processes that have a direct requirement for oxygen are more sensitive to oxygen deprivation than processes which are dependent upon oxygen supply because of redox-dependent reactions, and redox-dependent reactions are more sensitive to oxygen deprivation than are systems that require oxygen for production of high-energy bonds.

O_2-dependent drug oxidations. A comprehensive list of oxidases and oxygenases was prepared in 1978 by Keevil and Mason [5]. Any of these enzymes could function in drug metabolism providing that the drug is a substrate for that enzyme. In practical terms, however, the list is restricted by the narrow range of substrate specificities of many enzymes, such that a relatively small number of enzymes with broad substrate specificities catalyze most drug oxidations. A group of enzymes, generically termed cytochrome P-450, catalyzes diverse oxidations of xenobiotics and has been investigated intensively with regard to functions of the enzymes in drug metabolism. They are not unique in this function and several other oxidases are known to be involved in drug metabolism. The effect of hypoxia on these systems depends upon their $K_{m(O_2)}$ relative to the oxygen concentration to which they are exposed *in vivo*.

In the reaction cycle of the cytochrome P-450 system, substrate binding alters the spin state of the heme iron [6]; the equilibrium of spin states is affected by the lipophilicity of the substrate. Since the heme is also the binding site for O_2, it is expected that the nature of the substrate also determines the apparent affinity of the system for O_2 binding. Reported values substantiate that the apparent $K_{m(O_2)}$ varies with substrate even with the same form of cytochrome P-450 [7]. In mammalian species, the situation is complicated by the occurrence of multiple forms of the enzymes in the same membranal frac-

tion; these forms may have different $K_{m(O_2)}$ values with the same substrate. With different substrates in systems containing multiple forms of cytochrome P-450, reported values range from 0.7 μM (0.5 Torr) to 200 μM (140 Torr) [7–12]. Since the average hepatic oxygen concentration is about 35 μM [13], any substrate that has a K_m above about 10–15 μM should be dependent upon oxygen concentration even in normoxia. At present, most of the substances that have been studied are model compounds. Of more than forty reported values for $K_{m(O_2)}$, 25 per cent are greater than 10 μM and 25 per cent are in the range of 4–10 μM. From these studies, one can predict that *metabolism of a large number of therapeutic agents that are substrates for the cytochrome* P-450 *system will be decreased during even mild hypoxia.*

Microsomal amine oxidase is an FAD-containing enzyme that catalyzes mixed-function oxidations of numerous amines and hydrazines, including compounds such as the tricyclic phenothiazine drugs (see Ref. 14). Oxygen dependence studies by Poulsen and Ziegler [15] observed $K_{m(O_2)}$ values of 24.9 μM with trimethylamine as substrate and 21.0 μM with methimazole as substrate. Thus, as with the cytochrome P-450 system, *drug metabolism by microsomal amine oxidase appears to be oxygen-limited under physiologically important oxygen concentrations.*

Mitochondrial monoamine oxidases are peptidyl-linked FAD-containing enzymes that exist in at least two forms and catalyze the oxidative inactivation of neurotransmitter amines [16, 17]. Oxygen dependence studies of these activities have been studied with benzylamine, β-phenethylamine, tyramine and 5-hydroxytryptamine, with $K_{m(O_2)}$ values ranging from 0.23 to 5.5 mM O_2 [18–20]. These values suggest a marked dependence upon oxygen concentration *in vivo*. Tipton [18] discussed these high values with regard to *in vivo* function and noted that, because of the "ping–pong" mechanism, the relative catalytic efficiency of the enzyme is greater at low oxygen concentrations than at high concentrations. The important feature, however, is that the actual enzymic turnover under these conditions is dependent upon oxygen concentration.

A variety of other amine oxidases are known and may function in drug metabolism. Beef plasma amine oxidase was found to have an apparent $K_{m(O_2)}$ of 34 μM with 3.3 mM benzylamine and 6.7 μM with 0.3 mM benzylamine [21]. Pig plasma amine oxidase has an apparent $K_{m(O_2)}$ of 83 μM with saturating concentration of benzylamine [22]. Human placental diamine oxidase has an apparent $K_{m(O_2)}$ of 290 μM [23]. Indoleamine-2,3-dioxygenase was found to have an apparent $K_{m(O_2)}$ of 87 μM, measured by the oxygen dependence of the formation of the enzyme·tryptophan·O_2 complex [24].

Aldehyde oxidase and xanthine oxidase are enzymes that contain molybdenum, FAD, and four iron–sulfur centers and that catalyze the oxidation of compounds represented by the general formula, $R_1—CH=R_2$ [25]. They function in drug metabolism by oxidizing polar aromatics such as the nitrogen-containing heterocycle, allopurinol. Oxygen dependence studies of xanthine oxidase from milk gave values ranging from 24 to 240 μM [26–29]. Data of Handler *et al.* [30] allow calculation of a value of 23 μM for the $K_{m(O_2)}$ of aldehyde oxidase. Consequently, it must be presumed that drugs oxidized by either of these enzymes will show a dependence upon oxygen concentration *in vivo*.

The list of oxidases that function in drug oxidations will continue to expand as medicinal chemists develop agents that are closer structural analogs of biomolecules and thus act as substrates for some of the more specific oxidases. The examples of oxygen affinities given here for drug-metabolizing enzymes are similar to the oxygen affinities of other oxidases and, therefore, many drugs that are developed in the future can also be expected to be metabolized in an oxygen-dependent manner.

Redox-dependent drug metabolism. Other drugs are metabolized by pathways that are only indirectly dependent upon oxygen concentration. The reactions involved may be either oxidations or reductions and may be coupled to any system in which the intracellular potential is dependent upon the continuous removal of reducing equivalents by the transfer to oxygen. Most redox-dependent enzymes utilize NADH or NADPH as reductants or NAD^+ or $NADP^+$ as oxidants. Other cofactors can also be utilized, such as GSH, lipoate or pteridine, but the reduction states of these are also coupled to the pyridine nucleotide pools. Although transfer of electrons between the NADH/NAD^+ pair and the NADPH/$NADP^+$ pair is an isoenergetic process, the mitochondrial membrane is impermeable to these compounds, and estimates of the relative redox potentials indicate that the NADH/NAD^+ couple is not in equilibrium with the NADPH/$NADP^+$ couple in either the mitochondria or cytoplasm [31]. Ballard [32] studied the change in cytoplasmic NADH/NAD^+ and mitochondrial NADH/NAD^+ in rat liver as a function of inspired oxygen. The results demonstrate that the NADH/NAD^+ ratios in both subcellular fractions respond similarly to hypoxia and that an increased ratio is seen at an inspiratory oxygen content of less than 10 per cent. These values can be related to the cellular O_2 concentration by comparison to studies on isolated hepatocytes [8] in which the cytoplasmic NADH/NAD^+ ratio is increased below 30 μM O_2. Half-maximal change occurred at about 13 μM. Thus, reactions that are dependent upon the redox state of NADH will show altered metabolism over this range.

Reactions dependent upon the cellular redox state may be either oxidations or reductions, but oxidations are much more common. The most extensively studied reactions of this type are the NAD^+-dependent dehydrogenations involved in metabolism of ethanol and other alcohols. The oxidation of ethanol is limited by the reoxidation rate of NADH [33] and, hence the increased NADH/NAD^+ ratio that occurs during hypoxia can be expected to inhibit ethanol oxidation. Aldehyde oxidations occur by NAD^+-dependent aldehyde dehydrogenases as well as by the oxidases discussed above (aldehyde oxidase and xanthine oxidase). The half-reduction potentials of the alcohol/aldehyde pair and the aldehyde/acid pair are of great importance in determining the effect of hypoxia on the rates of the metabolic conversions.

The half-reduction potential for ethanol/acetaldehyde is about −200 mV, while the NADH/NAD^+ potential in the cytoplasm estimated from the lactate/pyruvate ratio is −250 mV. Thus, the cell will have a tendency to maintain a rather high ethanol/acetaldehyde ratio. As NADH/NAD^+ is increased during hypoxia, the ethanol/acetaldehyde ratio should be even higher. Thus, it is the highly negative half-reduction potential of the acetaldehyde/acetate couple (−600 mV) that allows the oxidation of ethanol in the cytoplasm. Secondary alcohols can be oxidized only to the ketone. Because the half-reduction potentials of these compounds are frequently positive relative to the pyridine nucleotide potential in the cytoplasm, metabolism of the ketones by the dehydrogenases involves reduction to the secondary alcohol.

A variety of other reductions of drugs occur, such as nitro- and azo-compounds to amines and disulfides to sulfhydryls. These reactions are usually dependent upon NADPH, and at present the effect of hypoxia on these reactions is not known.

Certain Phase II reactions of drug metabolism can be expected to be affected by hypoxia due to their dependence upon the cellular redox state. The most important general class of these reactions is glucuronide conjugate formation. Glucuronidation occurs by a group of enzymes (UDP glucuronyl-transferases) that utilize UDP glucuronic acid as substrate. UDP glucuronic acid is derived from glucose-1-phosphate and UTP in a two-step enzymic process. The UDP glucose that is formed in the first step is oxidized to UDP glucuronic acid in an NAD^+-dependent process. A direct study of the oxygen dependence of glucuronidation is not available; however, studies of the interaction of ethanol metabolism and drug conjugation indicate that glucuronidation is inhibited dramatically by ethanol [34]. This indicates that the rate of the overall glucuronidation process in liver cells is rate limited by the NAD^+-dependent reaction. Because of the known change in NADH/NAD^+ during hypoxia, it appears likely that glucuronidation will also be inhibited by hypoxia.

Many cellular oxidation reactions have the paradoxical feature that they require a reducing agent, such as NADPH, in addition to oxygen. These reactions are catalyzed by mixed-function oxidases [35]. For this type of reaction, which includes both cytochrome P-450 and microsomal amine oxidase, the effect of hypoxia *in vivo* might be a combination of inhibition, due to deficiency of oxygen directly as a substrate, and stimulation of activity due to increased NADPH. This possibility has not been examined, but Shigematsu *et al.* [36] found that the oxygen dependence of cytochrome P-450 is altered by NADPH or NADH supply. This may be of minor importance during mild hypoxia, however, since limitation of oxygen as substrate is likely to precede the increase in NADPH; therefore, an increased rate due to increased NADPH is unlikely.

Energy-dependent drug metabolism. Drug conjugation reactions are synthetic reactions that usually require a source of energy supplied directly or indirectly as ATP. These reactions include the formation of glucuronides, ethereal sulfates, mercapturic acids, glycine conjugates, glutamine conjugates, methylated products, and acetylated products. Because of the decreased ability to synthesize ATP during hypoxia, all of the processes are expected to be inhibited during severe hypoxia. The oxygen dependence of supply of the high-energy phosphoryl group has been studied for liver [8, 32] and brain [37]. Results indicate that the critical [O_2] is about 20 μM with a half-maximal change at about 1–2 μM. The higher oxygen concentrations of this oxygen-dependent range occur during mild hypoxia, and thus reactions with a high K_m for ATP (or corresponding high-energy component) may be inhibited under mild hypoxia.

The selectivity with regard to which conjugation reactions will decrease first depends upon the relative affinities of the different systems for ATP. As the ATP supply becomes limiting, the systems with the greatest affinity for the ATP will be spared at the expense of those systems with a poorer affinity. Among the systems listed above, those for sulfation and methylation have poorer affinities for ATP than do the systems for glucuronidation, glutathione conjugation, and acetylation. Sulfation requires ATP for the synthesis of active sulfur in the form of PAPS (3′-phosphoadenosine 5′-phosphosulfate). The apparent K_m for ATP is 1.6 mM [38]. Methylation involves *S*-adenosylmethionine which is formed from methionine and ATP by a reaction catalyzed by methionine adenosyl transferase. The apparent $K_{m(ATP)}$ for this enzyme is 2.6 mM [39]. The apparent K_m values for the two reactions that require ATP in synthesis of GSH, γ-glutamylcysteine synthase and GSH synthase, are 0.2 and 0.1 mM respectively [40, 41]. The apparent K_m values for the two reactions involved in supply of cytoplasmic acetyl-CoA, mitochondrial pyruvate carboxylase and ATP-citrate lyase, are 0.058 and 0.28 mM respectively [42, 43]. The energy-requiring step in glucuronidation is the UTP-dependent UDP-glucose pyrophosphorylase which has an apparent $K_{m(UTP)}$ equal to 0.2 mM [44].

In vivo *considerations.* The above information, obtained from biochemical studies of purified enzymes, subcellular fractions and isolated cells, clearly indicates that drug metabolism is dependent upon oxygen supply and that metabolism of different drugs will be differentially affected by the severity of hypoxia. Similar studies on perfused organs and with intact animals have been limited mostly to the metabolism of hexobarbital and pentobarbital [45–51]. The results of these studies show that in acute hypoxia the rate of disappearance of hexobarbital and pentobarbital from the blood is decreased [47–51], but that following prolonged hypoxia (5 days), the rate of disappearance in animals re-exposed to normoxia is increased relative to those that have been maintained under normoxia [45–47]. These results suggest that the decreased oxygen supply during acute hypoxia results in decreased metabolism in the same manner as seen in the cellular and subcellular systems. In these studies, however, other factors such as renal clearance were not taken into account. An increased rate of metabolism following chronic hypoxia suggests that hypoxia induces the drug-metabolizing systems. Other studies [52, 53] support this conclusion and lead to the possibility that effects on drug metabolism in chronic hypoxia

are different than in acute hypoxia due to alterations in concentrations of relevant enzyme systems.

Additional complications arise from the difficulty in defining hypoxia on a cellular basis, the difficulty in obtaining a correct metabolism rate *in vivo*, and the difficulty in developing a perfusion system that adequately reflects the conditions of oxygenation that occur *in vivo*. The arterial P_{O_2} is a valuable measurement but may be misleading with regard to the severity of hypoxia at the cellular level because of compensatory mechanisms that enhance net blood flow per unit tissue mass. In addition, arterial measurements do not assess organ supply and may not detect regional hypoxias. A suitable model to assess the oxygen dependence of drug metabolism may be perfused organ systems such as the perfused liver. Such application has been made but usually provides perfusion only through the portal vein. Although the majority of blood flow occurs by this route, the oxygen content of portal blood is much lower than that of hepatic arterial blood, such that the arterial blood supply may be important in determining the adequate oxygenation of the liver. In addition, perfusion with hemoglobin-free medium greatly increases the degree of tissue oxygen gradients because of the decreased oxygen carrying capacity.

Finally, measurements of the effects of hypoxia on drug metabolism must involve measurements of rates of drug metabolism and not indirect measures such as pharmacological or physiological actions. Of course these latter actions are of utmost importance to the medical use of drugs during hypoxia, but to understand the mechanisms of altered drug actions, it is necessary to distinguish between altered metabolism rates and other factors. Serum drug concentrations are not determined simply by the rate of metabolism but are also dependent upon uptake, distribution, and excretion. Each of these factors can also be modified during hypoxia, especially due to the variety of physiological adaptations (increased cardiac output, opening of non-functional capillaries, vasodilation, hemoconcentration, etc.) that occur. To understand the effect of hypoxia on drug metabolism *in vivo* will require control for these other factors.

In conclusion, a large body of biochemical data on drug-metabolizing systems indicates that metabolism of a variety of drugs is oxygen dependent under hypoxic conditions. At present, no generalizations can be made to allow one to readily recognize which drugs will be affected, but those metabolized directly by oxidases and oxygenases and those conjugated as ethereal sulfates, glucuronides, or methylated derivatives should be studied in more detail. *In vivo* studies must be designed so that other factors affecting pharmacologic activity are controlled, and ideally, drug metabolism *in vivo* as a function of oxygen supply should be measured directly.

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