**Metalloporphyrins as chemical mimics of cytochrome P-450 systems**

The liver, in humans and animals, naturally metabolizes most drug molecules through oxidative mechanisms into metabolites. The formation of these metabolites is catalyzed predominantly by cytochrome containing enzymes bound with a heme cofactor. The most familiar of these enzymes responsible for catalyzing a range of biological oxidations is the P-450 mono-oxygenase. In all of these heme-containing proteins which includes catalases, peroxidases and ligninases, the proteins are activated by hydrogen peroxide, and catalysis is initiated via a two-electron oxidation of the ferric resting state to an oxoferryl porphyrin cation radical (I).



While the oxidation state for the cytochromes P-450 has yet to be characterized, most of their reactions and those of the biomimetic analogs can be accounted for by an oxygen transfer from (I) to a variety of substrates to give characteristic reactions such as hydroxylation, epoxidation and heteroatom oxidation.Other products resulting from hydroxyl and hydroperoxyl radicals have also been detected. The metabolic processes *in vivo* contribute substantially towards the efficacy, side effects and toxicity of the pharmaceutical entity. These factors are key in the success or failure of a clinical candidate therefore the metabolic processes of drug molecules in the body are always the subject of intense scrutiny in pharmaceutical companies.

Toxicological and pharmacological studies on the metabolites form a crucial segment in the identification of a clinical candidate. Traditionally pharmacologists have been involved in the isolation and identification of the metabolites of a drug. It is imperative that metabolite studies are conducted early in the drug development process.

**Current issues:**

Several issues of contention have come to attention with the use of biological systems in studying drug metabolism:

1. *In vitro* studies produce very small quantities of the primary metabolites, which are often hydrophilic and difficult to isolate.
2. Animal studies necessitate the sacrifice of animals and are extremely expensive to conduct. Liver slice preparations are of variable potency; it is difficult to quantitate the precise stoichiometry of the oxidant.
3. Pharmacologists have no prior knowledge of the metabolite structure they need to seek.
4. Many of the metabolites are not amenable to organic synthesis by conventional routes.

We therefore turned to the metalloporphyrins as mimics of the in *vivo* metabolic processes.

**The oxometalloporphyrins:**

Synthetic metalloporphyrins have recently received a lot of attention as mimics of numerous enzymes, specifically 10 models for peroxidases and particularly the ligninases. Metalloporphyrins have also found utility as model systems for studies of the oxidative metabolism of drugs. A detailed study of the metabolism of lidocaine has been reported as have preliminary studies on the use of metalloporphyrins as chemical mimics of cytochrome P-450 systems.78

The first synthetic metalloporphyrins were found to be oxidatively labile. Few catalytic turnovers were seen due to the rapid destruction of the porphyrin macrocycle. Dolphin showed that the introduction of halogen groups onto the aryl moeity (of meso-tetraarylporphyrins) and on the β-pyrrolic positions of the porphyrins increased the turnover of catalytic reactions by decreasing the rate of porphyrin destruction. In addition, the combined electronegativities of the halogen substituents are transmitted to the metal atom making the corresponding oxo-complexes more electron deficient and thus a more effective oxidation catalyst.

**Application of the Methodology to Selected Drugs:**

We have recently applied this technique to study the results of oxidative metabolism on several drugs. Reactions using our metalloporphyrins were conducted in methylene chloride/water or water/acetone to nitrile (80:20, v/v) at ambient temperatures. Iodosobenzene, cumene hydroperoxide, hydrogen peroxide or sodium hypochlorite were used as the source of exogenous oxygen. Products were separated by HPLC and identified by comparing authentic reference samples of the major metabolites of these drugs previously isolated from the urine of rats or characterized from rat liver microsomal incubations.









The oxidation of aminopyrene was particularly interesting in that new, hitherto unobserved metabolites were detected. The variation of the reaction conditions led to the synthesis of selected metabolites in increased yields.



This was also exemplified by the oxidation of lidocaine; two new oxidation products were observed.



These two oxidation products have escaped detection in all previous studies.

It is useful to note that these are rare examples of porphyrin-mediated oxidation of sophisticated pharmaceutical entities. The reactions described above are generally applicable and have been used in our laboratories to achieve hydroxylation and *N*-demethylation reactions on numerous other substrates. The hydroxyl metabolite and the desmethyl derivative were usually difficult to synthesize by conventional organic chemistry methods.

This approach affords an efficient method for the systematic preparation and identification of the entire spectrum of metabolites from a chosen drug. We can envision the screening of a series of compounds by reacting them with different permutations of a metalloporphyrin, co-oxidant and a suitable solvent. Reaction conditions can be refined to produce the maximum number of metabolites. This logically leads to subsequent scaled-up optimal processes. The oxidation products can be separated and subjected to toxicological, pathological, histopathalogical, or gentoxic testing.