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# Drug Targeting Using Bioreductive Delivery Systems

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Hypoxia (low oxygen concentration) is associated with the abnormal physiology found in a number of diseased tissues. These include the inflamed synovial membrane in rheumatoid arthritis, solid tumours in cancer and peripheral tissues in uncontrolled diabetes. This pathological physiology is being exploited for the development of bioreductive drug targeting systems. This review will focus on the three major classes of bioreductive delivery systems developed to date: Nitroheterocycles, Indolequinones and Cyclisables. In the first system, the variety of known nitroheterocycles facilitates the development of a wide range of delivery vehicles. Thus, various nitroimidazole-, nitrofuran- and nitrothiophene- drug conjugates have been prepared to exploit the wide range of reduction (activation) potentials of these heterocycles. For the indolequinones, vigorous research activity has resulted in the preparation of a wide range of analogues based on the original mitomycin C structure. For the cyclisable bioreductive delivery agents, concomitant intramolecular cyclisation is a feature of the drug release process. Two cyclisation-driven systems have been reported and are based upon i) self-alkylation and ii) through-bond cyclisation. Development of the cyclisables is of particular interest, as it will facilitate "inert" delivery to hypoxic tissues without interaction with DNA as was expected with the nitroheterocycles and indolequinones. The current vigorous activity in developing a broad range of bioreductive delivery agents, coupled to the wide range of diseases where hypoxia is a feature, warrants an optimistic outcome for this field.

Key Words: **Drug delivery, Prodrug, Nitroheterocycle, Indolequinone, Cyclisable, Reductase, Hypoxia, Cancer, Rheumatoid arthritis, Diabetes**

## Introduction

Selective drug delivery systems are frequently based upon a pathological characteristic of the diseased tissue of interest. Tissue hypoxia (an inadequate oxygen content) is a characteristic feature of a wide range of diseases, can be chronic or acute and has been the subject of vigorous research activity over the past three decades. In particular, drug delivery based upon bioreductives that are activated by reductase enzymes to release the active drug has received considerable interest. The selectivity for hypoxia is afforded by the reversal of the first reduction step by oxygen in a process that gives the parent conjugate. Thus, in normoxic tissues, re-oxidation by oxygen to the parent conjugate hampers reductively activated release of the drug.

The principles of bioreductive-based therapies grew from the isolation of bioreductive cytotoxics from microbes and their initial use as anti-microbial agents. Further development has led to their incorporation as anti-cancer prodrugs capable of killing tumour cells upon activation at hypoxic loci in solid tumours (Rauth et al. 1998). Both nitroheterocycles and indolequinones form adducts with DNA after they are reduced in hypoxic tissues (Varghese et al. 1980, Tomasz et al. 1994). The considerable effort expended in developing bioreductives as cytotoxic prodrugs has led to an

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immense technological platform for the development of associated bioreductive delivery systems. Thus, the principal moieties used as bioreductive cytotoxics (nitroheterocycles and indolequinones) have undergone development as drug delivery agents. However, as both of these moieties are, in principle, capable of cytotoxic activity, their use as delivery agents is somewhat restricted and they are most suitable for delivery in life-threatening diseases, such as cancer, where the use of the cytotoxic is warranted. This limitation has led to the development of self-inactivating bioreductive delivery systems where drug release occurs concomitantly with cyclisation of the bioreductive remnant. This cyclisation process is designed to remove the capacity for DNA adduct formation and thus render "inert" the reduced residue.

The principal underlying bioreductive delivery systems depends on the stability of the conjugate in normoxic tissues and the release profile upon activation in hypoxia. Thus, the cardinal feature required for successful delivery is the trade-off in stability of the conjugate versus drug ejection upon reductive activation. A key feature that dictates the balance between stability and drug release is the reduction potential of the bioreductive moiety. A reduction potential of ca. -350 mV is generally considered to offer the best trade-off for targeting hypoxic mammalian tissues. Of the the bioreductive systems studied to date, examples of the indolequinone, nitroimidazole and nitrofuran classes exhibit reduction potentials in this vicinity.

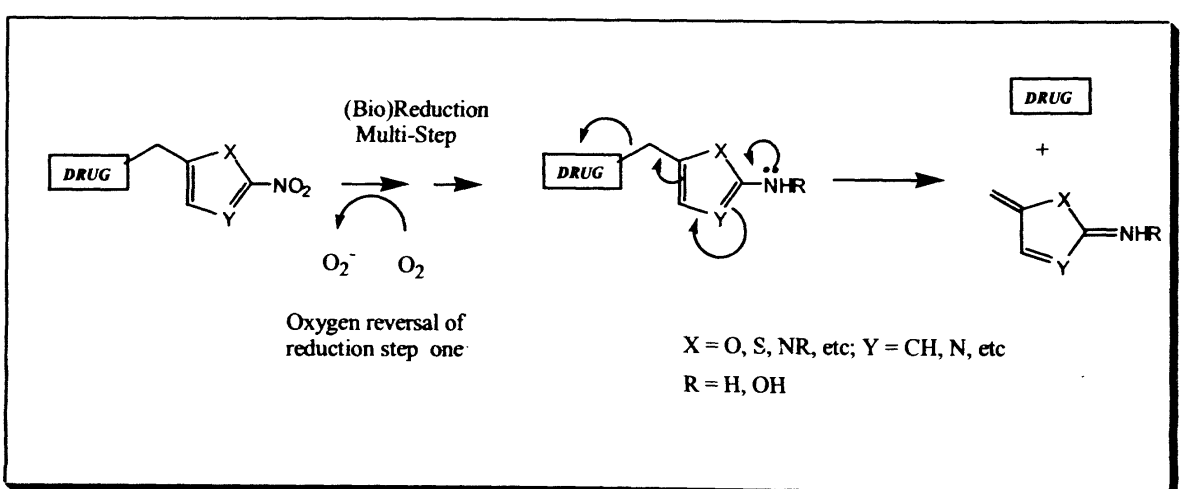
Increased interest in the role of hypoxia in disease has led to a significant increase in the number of diseases where hypoxia is suggested to play a role. These include some cancers, rheumatoid arthritis, diabetes, renal disease and some cardiovascular diseases. Hypoxia-based therapeutic strategies are gaining importance as an increasing number of roles of hypoxia in biological systems are delineated. Hypoxia has been shown to control the regulation of enzyme expression at the transcription level via hypoxia-inducible factors, which results in the up-regulation of key reductase enzymes required for the activation of bioreductive delivery systems. Thus the exploitation of hypoxia has a dual advantage in that (i) the increased levels of reductases lead to enhanced activation, and (ii) the reduced concentrations of oxygen facilitate the reduction process.

In this review, we will outline recent progress in the development of the three major classes of bioreductive delivery systems. Our discussion will entail the major challenges and future perspectives and will include the drugs that have been conjugated to the bioreductives.

## Delivery Systems

### *Nitroheterocycles*

In the past five years, bioreductive-drug conjugates based on nitrofurans, nitroimidazoles and nitrothiophenes have been reported (Berry et al. 1997, Parveen et al. 1999, Everett et al. 1999, Ferrer et al. 2001). In all cases, the active drug was released




**Scheme1** Proposed mechanism for bioreductive activation of nitroheterocycle-based drug delivery vehicles. The reversal of the first reduction step by oxygen (in normoxic tissues) regenerates the parent conjugate, preventing release of the drug.

by reduction in model systems, indicating their potential as delivery agents (Scheme 1). These reports describe the conjugation of a non-steroidal anti-inflammatory agent (NSAID), a corticosteroid and inhibitors of poly(ADP-ribose)polymerase (PARP) to nitroaromatic heterocycles and subsequent drug release in vitro in model chemical reduction systems.

Nitroheterocycles have a number of advantages for use in the development of bioreductive delivery systems. They are commonly used as anti-microbial agents and, in many cases, have a limited side-effect profile. They vary greatly in their one-electron reduction potentials, which allow their adaptation for targeting drugs to mammalian tissues, in addition to their application in targeting microbes. In a number of conjugate types, they have been shown to release the active drug, despite having a relatively stable linker bond (such as carbon-nitrogen) (Parveen et al. 1999). Thus, the crucial trade-off between stability of the conjugate and drug release upon activation in hypoxia is realistically achievable for this class of bioreductive.

### *Indolequinones*

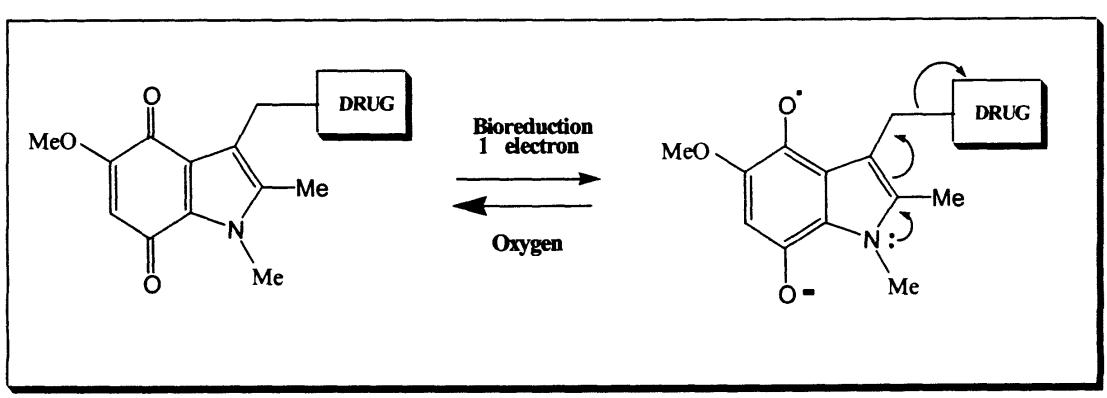
The development of indolequinones as bioreductive delivery agents grew from research on the use of mitomycin C as an anti-cancer agent. The one-electron reduction potentials of indolequinones are ideal for targeting hypoxic mammalian tissues (Scheme 2). A number of varied structural analogues of the base indolequinone structure have been prepared. These analogues have been conjugated to a wide range of drugs, including corticosteroids and other anti-inflammatory agents (Ferrer et al. 2000, Jaffar et al. 1999), anti-cancer agents (Ferrer et al. 2002a) and inhibitors of PARP (Ferrer et al. 2002b). In addition, numerous model compounds have been prepared to optimise rates of drug release and elucidate the mechanism of release (Everett et al. 1998, Jaffar et al. 1998, Everett et al. 2001, Swann et al. 2001).

### *Cyclisables*

Two classes of delivery vehicle are under development to deliver drugs to hypoxic tissues via an "inert" bioreductive carrier. The 'self-alkylating' and 'through bond' cyclisation processes permit the release process with intramolecular cyclisation occurring at the bioreductive unit. This intramolecular cyclisation deactivates the bioreductive during drug release to avoid deleterious adduct formation with macromolecules such as DNA. Negation of the adduct-forming capacity of the bioreduced unit after drug release is ideal for delivery in a wider variety of diseases where the cytotoxic aspects of the bioreductive are contraindicated.

### Self-alkylating Bioreductives

For treatment of many diseases it is desirable to deliver drugs with essentially an inert delivery system. For "inert" delivery of drugs to hypoxic tissues via bioreductive vehicles, it is essential to overcome adduct formation between the bioreductive remnant and DNA. The self-alkylating delivery concept was designed to incorporate a nucleophile within the bioreductive that preferentially reacts with the activated bioreductive remnant during or after release of the drug. The reduction driven intramolecular cyclisation functions to (i) assist drug release and (ii) stabilise the bioreductive remnant and remove the potential for DNA adduct formation.

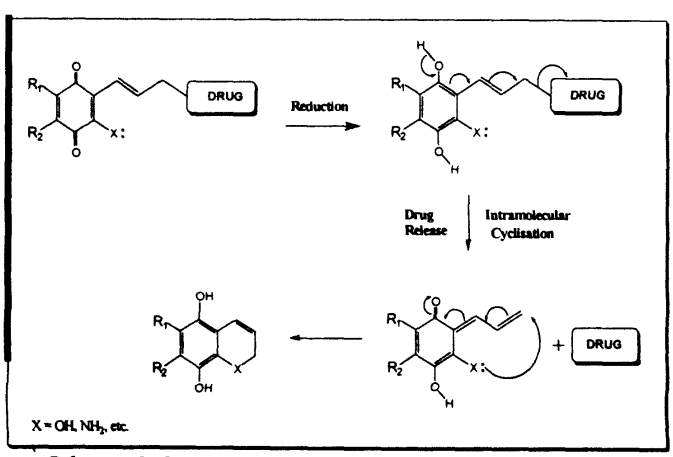



**Scheme 2.** Proposed mechanism for bioreductive activation of indolequinone-based drug delivery vehicles.

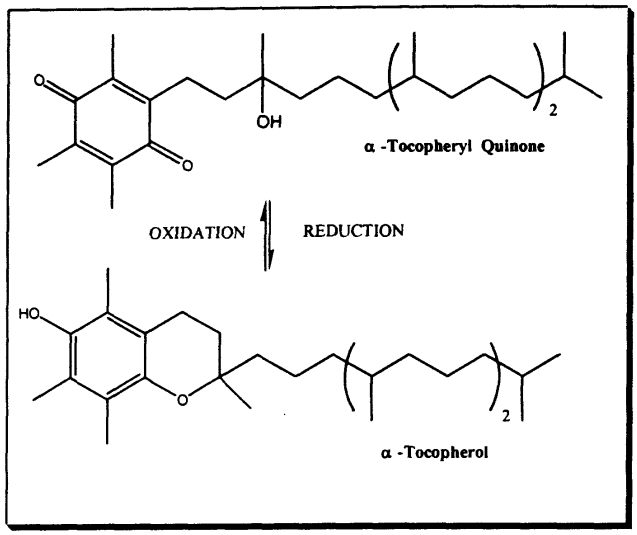
The self-alkylation process is shown diagrammatically in Scheme 3 for a quinone alkenyl analogue. To optimise the efficacy of the self-alkylation process, design features include optimizing ring size and imparting steric hindrance to facilitate ring closure and protect against intermolecular reactivity with DNA. These delivery systems are currently under development (Naughton et al. 1998).

A search for natural self-alkylating compounds, to minimise potential side effects, resulted in the development of vitamin E analogues for bioreductive drug delivery (Naughton et al. 2000, Naughton 2001). Oxidation of vitamin E results in ring opening from the tocopherol form to generate the tocopheryl quinone (Scheme 4). This oxidation-driven ring opening is accompanied by reversible expulsion of the alkoxide. Reduction of oxidised vitamin E (tocopheryl quinone TQ) results in cyclisation, with concomitant ejection of an hydroxy group (Scheme 4) (Liebler 1993, Moore et al. 1997, Wijesundara et al. 1994). Hypoxia-selective targeting based on this system is under development and has been reported (Naughton et al. 2000, Naughton 2001).

Current development of the vitamin E system is centred on analogues of Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E. The oxidised form of Trolox® has been conjugated to acetylsalicylic acid via the hydroxy group (Scheme 5). Reduction in chemical model systems resulted in cyclisation with concomitant release of the active drug.




Scheme 3. Diagrammatic representation of self-alkylation



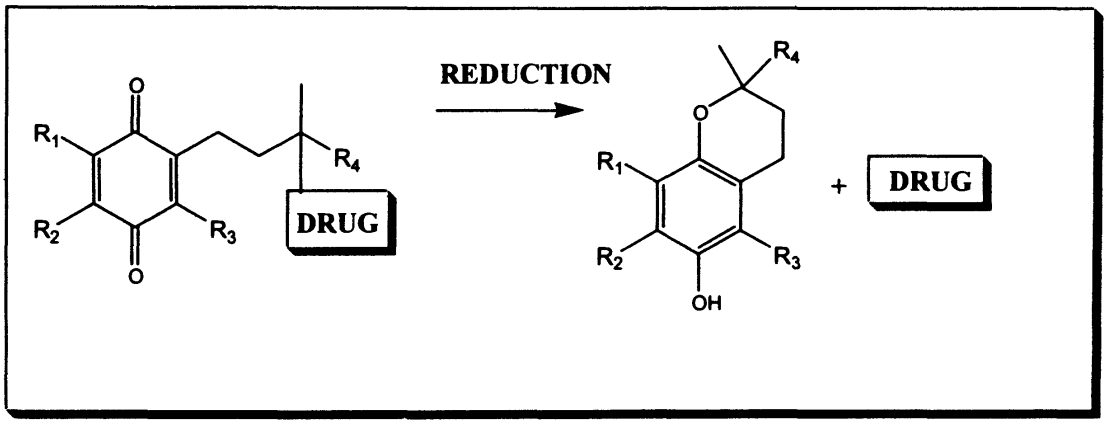

Scheme 4. Redox driven cyclisation and ring opening of vitamin E.

*Through bond cyclisation*

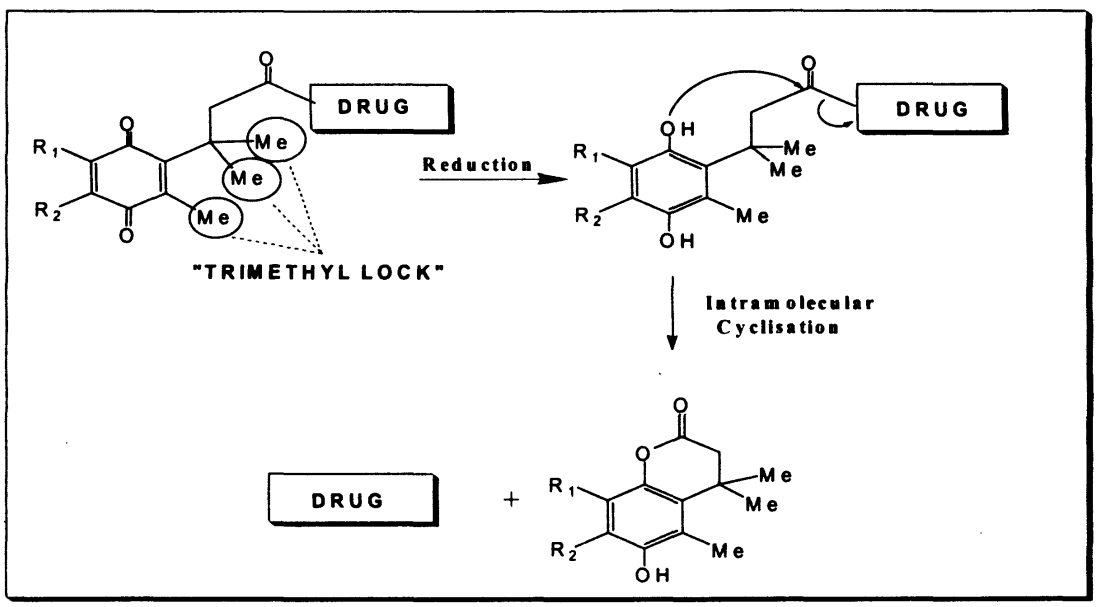
A lactonization-based system has been described which incorporates steric hindrance to enhance the rates of ring closure and accompanying drug ejection (Scheme 6). In this system the benzoquinone acts as a trigger and the propionic moiety acts as the linker while a trimethyl lock provides the steric hindrance necessary to optimise ring closure (Amsberry et al. 1991, Chikhale et al. 1997). Steric hindrance imparted by the methyl groups (termed "trimethyl lock") has been shown to significantly facilitate lactonization (Wang et al. 1996).

The quinone propionic delivery system has been conjugated to melphalan [4-[bis(chloro-2-ethyl)amino]-L-phenylalanine], an alkylating agent. It has been shown to cross cell membranes and accumulate intracellularly in a human adenocarcinoma cell line (Gharat et al. 2001). Further work is necessary to acquire preferential delivery to particular tissues under hypoxia. This may require 'tuning' the reduction potential by chemical modification of the simple benzoquinone to target hypoxic tissues selectively.

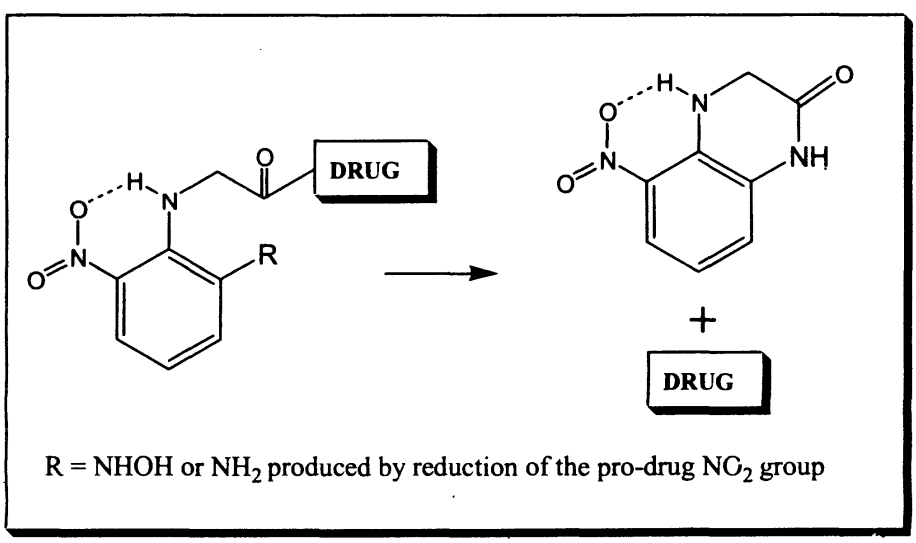
An alternative through-bond cyclisation approach involving amine formation is under development (Sykes et al. 1999). In this delivery system, a N-(2,6-dinitrophenyl)amino group acts as the bioreductive. Reduction of the nitroaromatic group drives cyclisation with concomitant extrusion of the active drug. Upon reduction of the nitro moiety to an hydroxylamine, the drug is released by nucleophilic attack on the adjacent amide carbonyl group (Scheme 7). The second nitro group directs the cyclisation process by 'locking' the carbonyl in a




Scheme 5. Bioreductive delivery based on the vitamin E analogue Trolox®.




Scheme 6. Bioreductive delivery using the "through bond" quinone-based delivery system




Scheme 7. Bioreductive delivery using the "through bond" nitroaromatic-based delivery system

favourable position through steric hindrance and hydrogen bonding with the anilino NH group. The second nitro group also raises the reduction potential to favour drug release in mammalian cells. A series of model compounds have been studied in vitro to optimise drug release (Sykes et al. 1999). Prodrug analogues of mustard and indoline alkylating agents were prepared (Sykes et al. 1999). However, these prodrugs were inefficiently activated by cellular reductases (Sykes et al. 1999).

## Conclusions and Future Directions

The widespread presence of hypoxia in diseased tissues, coupled to its upregulation of reductase enzymes, affords exciting opportunities to overcome drug side effects through bioreductive drug delivery. To date, a number of limitations exist which restrict the use of these delivery systems. Bioreductive delivery systems need to be optimised to ensure delivery is maximised to hypoxic tissues. To this end, extensive research is under way to enhance the stability and release parameters of drug conjugates of the three major classes of bioreductive. Development of model conjugates is aimed at enhancing the stability under normoxic 'non-reducing' conditions coupled with effecting drug release upon reduction under 'reducing' conditions in hypoxia. Much information regarding optimal types of linker groups and bonds is being gleaned from studies of both model compounds and drug conjugates.

For the non-cyclisable bioreductive delivery systems, their application may be limited to serious conditions like cancer where the DNA-adduct forming ability of the bioreductive remnant may be a bonus. Use of general nitroheterocycle- and indolequinone- based systems in diseases such as rheumatoid arthritis would be inappropriate owing to the possibility of this serious side-effect. For this reason the cyclisables have been introduced. Most examples of cyclisables reported to date are based on simple benzoquinoid moieties for exemplification in chemical model systems. The ease of reduction of these species would rule out their adoption for targeting hypoxic tissues. Thus, structural changes such as substitution of an indolequinone moiety for the benzoquinone remnant will be required for in vivo testing. Further work is under way towards optimising the reduction potential of the bioreductive moiety to enable satisfactory reductive activation selectively in hypoxic tissues.

The development of pharmacokinetically stable conjugates which undergo drug release upon reduction allow full release studies to be undertaken in vivo. These in vivo studies will allow generic parameters such as half-life, side-effect profiles and fates of bioreductive remnants after drug release to be ascertained. The ultimate goal is to develop a non-toxic delivery system suitable for oral administration.

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