Hypoxia: Targeting the Tumour

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Abstract: Solid tumours contain regions of very low oxygen concentrations that are said to be hypoxic. Hypoxia is a natural phenotype of solid tumours resulting from an imperfect vascular network. There are a number of consequences associated with tumour hypoxia including: resistance to ionising radiation, resistance to chemotherapy and the magnification of mutated p53. In addition tissue hypoxia has been regarded as a key factor for tumour aggressiveness and metastasis by activation of signal transduction pathways and gene regulatory mechanisms.

It is clear that hypoxia in solid tumours promotes a strong oncogenic phenotype and is a phenomenon that occurs in all solid tumours. As such this provides a significant target for drug discovery particularly for tumour-targeting agents. A range of chemical classes (N-oxides, quinones, nitro-aromatics) have been explored as bioreductive agents that target tumour hypoxia. The most advanced agent, tirapazamine, is in phase III clinical trials in combination with cis-platin. The aim of this review is to give a brief overview of the current molecules and strategies being explored for targeting tumour hypoxia.

Key Words: Hypoxia, tumour targeting, bioreduction.

INTRODUCTION

In 2004 there were 10 million new diagnosed cancer cases and this figure is expected to increase to 15 million by the year 2020 [1]. It is important to recognise that as this number increases, the need for viable treatments becomes ever greater. Although many clinically used chemotherapeutic agents today target specific molecular targets that are not unique to neoplastic cells and thus lead to adverse systemic effects, the shift towards drugs targeting a specific pharmacological abnormality of cancer can lead to very exciting opportunities for treatment. The current research trends and clinical data, including the success of Imatinib for the treatment of chronic myeloid leukaemia (CML) [2] and Herceptin for Her2+ve breast cancer [3] firmly establishes the value of such a targeted approach.

Whilst the existence of molecular abnormalities such as the chimaeric fusion protein BCR-Abl, which is constitutively active in CML, can provide a unique target for drug intervention, such clear targets may not always exist. However, there are some general phenotypes that also offer good opportunities for drug intervention. Tumour hypoxia, first recognised by Gray *et al*. in 1953 [4,5] is now well established as a key phenotype of solid human tumours [6] and its existence provides an exciting target for drug discovery.

TUMOUR HYPOXIA

The aggressive growth of solid tumours exerts pressure on surrounding vasculature to maintain a sufficient supply of blood and nutrients to these cells. The growing distance of cells from the blood supply (>150uM) ultimately leads to regions of very low oxygen concentrations that are said to be hypoxic (Fig. **1**) [7]. There are a number of consequences associated with tumour hypoxia including: resistance to ionising radiation [5], resistance to chemotherapy [8], induction of resistant mechanisms [9] and the magnification of mutated p53 [10]. One of the main mechanistic responses of a tumour cell to hypoxia is the induction of the hypoxia-inducible transciption factor 1 (HIF-1) [11]. HIF-1 has a key role in the expression of genes involved with glucose uptake, oxygen transport and angiogenisis [12]. Hypoxia-inducible factor 1-alpha (HIF-1α) protein over-expression has been shown in patients with invasive breast cancer and is associated with poor prognosis [13]. In addition, the response to radiotherapy has been linked to the over-expression of HIF-1 in addition to the oxygen fixation of radiation induced radicals [14].

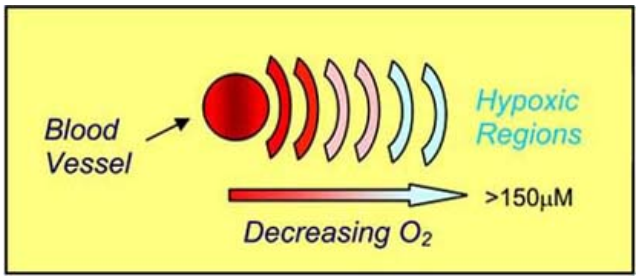

Fig. (1).

Whilst tumour hypoxia is a key factor associated with tumour aggressiveness and metastasis through activation of signal transduction pathways and gene regulatory mechanisms [15], its existence provides an opportunity that has been exploited by tumour targeting agents. The purpose of this review is to give an overview of the current molecules

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being developed which target tumour hypoxia. HIF-1 has been reviewed recently and will not be covered here [12].

DRUGS TARGETING HYPOXIA

The concept of designing molecules that are void of systemic toxicity until activated by tumour hypoxia has been investigated for many years and a number of excellent reviews have been published [16-18]. Although specific examples may differ, there are a number of essential requirements to fulfil: good diffusion in order to reach hypoxic tissues, an oxygen sensitive enzymatic reduction (bioreduction) to an active species and ideally diffusion of the active species to neighbouring tumour cells (i.e. the killing of aerobic tumour cells) known as a bystander effect.

There are currently at least six molecules undergoing clinical evaluation that are targeted to the hypoxic areas of solid tumours (Fig. **2**). The following section outlines the current status of these compounds and their potential value to the clinical oncologist.

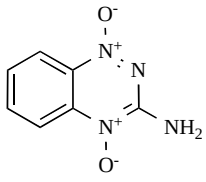
TIRAPAZAMINE

Tirapazamine (**1** - TPZ) [19] belongs to a class of aromatic *N*-oxides that was first designed to target hypoxic cells resistant to ionising radiation [20]. Of all the molecules that have been developed, TPZ is the most clinically advanced hypoxia activated agent to date, currently being evaluated in phase III clinical trials in combination with cisplatin for the treatment of patients with locally advanced head and neck cancer [21].

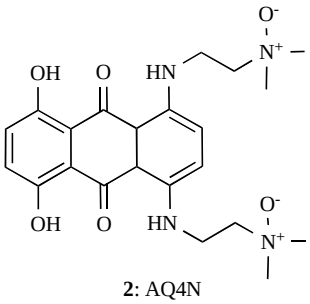
TPZ kills hypoxic cells by inducing DNA damage following an oxygen sensitive bioreduction. The clinical efficacy of TPZ is proposed to be due, in part, to its ability to kill tumour cells at intermediate oxygen concentrations [22]. The first step of this mechanism involves a one-electron reduction mediated chiefly by cytochrome P450 reductase. Under hypoxia, the resulting radical has been shown to dehydrate to an oxidising radical that is believed to be responsible for inducing DNA damage [23]. The primary metabolite, SR4317 (**7**) is thought to be important for fixing the initial DNA radical and has also been shown to potentiate the toxicity of TPZ [24]. Under aerobic conditions, the one-electron reduction product is rapidly back oxidised by molecular oxygen to the parent molecule, limiting toxicity in well oxygenated tissues (Fig. **3**).

A key parameter in the first step of this process is the one-electron reduction potential, *E(1)* [25]. These values are used to predict the potential selectivity of a bioreduction under hypoxia, where reduction potentials approaching -200mV (O_2 is -155mV) indicate potential aerobic reduction and potentials lower than -500mV suggest a slow reduction under hypoxia. In a study with TPZ and 34 analogues [26], the expected aerobic cytotoxicity correlated well with the *E*(1) values, but no clear relationship existed between the *E(1)* values and hypoxic selectivity against SCCVII cells (clonogenic assay) and none of the compounds tested displayed a hypoxic selectivity greater than TPZ itself. It is likely that the *E(1)* value for TPZ is optimal and *E*(1) values for optimal hypoxic selectivity have been shown to be between -450mV to -510mV with TPZ having an *E(1)* value of -456mV. It is clear that this parameter for all compounds activated by ubiquitous one-electron reductase is crucial for achieving hypoxic selectivity.

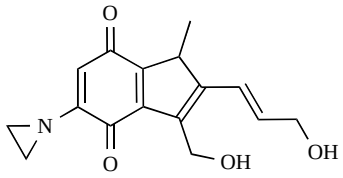
It is well known that the physico-chemical properties determine the pharmaco-dynamic properties of any given molecule [27]. In particular, penetration of anticancer drugs through tumour tissue has been associated with resistance to chemotherapy [28]. This is a primary concern for molecules targeting the hypoxic fraction of a tumour as these regions are the furthest from the blood vasculature. The superior



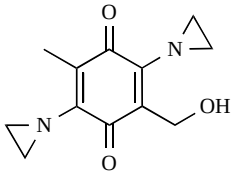
1: Tirapazamine



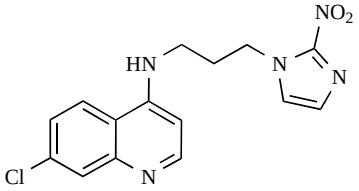
2: AQ4N



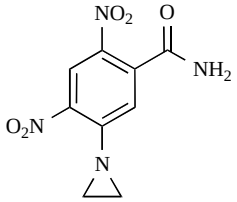
3: EO9



4: RH1



5: NLCQ-1



6: CB1954

Fig. (2).

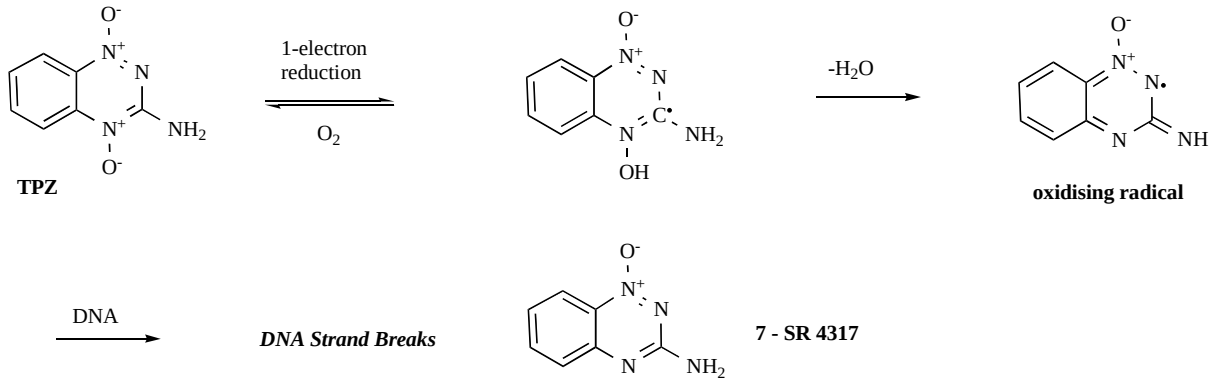


Fig. (3).

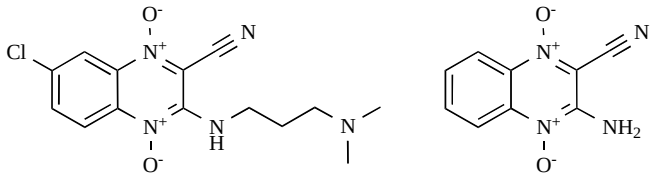
diffusion of the TPZ analogues (**8**) and (**9**) in HT29 multicellular layer cultures has been described [29] showing that increases in log *P* can lead to improved extravascular transport and are predicted to improve cytotoxicity against hypoxic cells in tumours (Fig. **4**).

Whilst TPZ fulfils many of the features required for a tumour activated agent, the oxidising radical responsible for the DNA damage does not produce a bystander effect and thus may limit its efficacy. However, in addition to the mechanism outlined above, TPZ has been implicated as a tumour activated topoisomerase II poison [30] and more recently has been shown to induce vascular dysfunction[1] and both may contribute toward the clinical benefit seen with TPZ.

The 3-amino-2-quinoxalinecarbonitriles TX-402 (**10**) [31] and Q-85 HCl (**11**) [32] are two molecules belonging to the same class of aromatic *N*-oxides (Fig. **5**). Recent studies in combination with the antivascular agent ZD6216 in human head and neck squamous cells have shown TX-402 to be superior to TPZ [33].

AQ4N

The bis tertiary amine *N*-oxide AQ4N (**2**) is currently in phase I/II clinical trials[2]. Unlike many other bioreductive agents, AQ4N undergoes a two-electron enzyme reduction mediated by members of the cytochrome P450 (CYP) family [34]. The resulting metabolite AQ4 (Fig. **6**) binds tightly to DNA and is a potent topoisomerase II inhibitor. The presence of oxygen diminishes the amount of AQ4N metabolised by the CYP family and thus results in selectivity for hypoxic tissues. Although hypoxia is common in all solid tumours, expression of CYPs can be variable and strategies to overcome this have focused on the use of gene-directed enzyme prodrug therapy (GDEPT) using both CYP3A4 [35] and CYP2B6 [36].

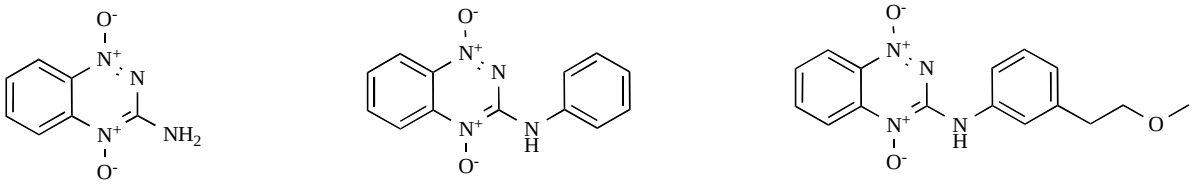


10: Q-85.HCl **11**: TX-402

Fig. (5).

EO9

EO9 (**3**) belongs to the quinone class of which the natural product mitomycin C and porfiromycin (Fig. **6**) were the first compounds to be clinically investigated as hypoxic selective agents [37]. As a class, these undergo both a one-electron reduction by p450 reductase (oxygen sensitive) and a two-electron reduction by DT-diaphorase (oxygen insensitive).



1: log P -0.34 **8**: log P 1.43 **9**: log P 1.51

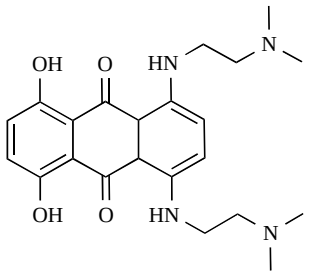
Fig. (4).

Whilst the two-electron reduction may limit hypoxic selectivity, DTD is often over-expressed in tumour cells and thus

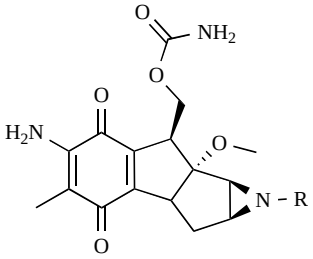
[1]Minchinton, A.I.; Huxham, L.A.; Baker, J.H.E.; McNicol, K.L.; Kyle, A.H. *The Tumour Microenvironment, 2005*, Oxford Aug 20-23. Conclusions were that hypoxic cell kill is *via* necrosis following TPZ induced vascular dysfunction.

[2]For more information see: www.novacea.com.

[3]Data presented at the Tumour–selective medicines conference organised by the Royal Pharmaceutical Society by Phillips, R. on 12th Oct 2005.



12: AQ4



13: R=H Mitomycin C
14: R=Me Porfiromycin

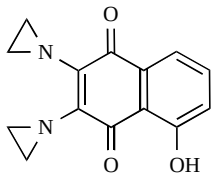


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Fig. (6).

still represents a tumour targeting mechanism [38]. Initial clinical trials with EO9 failed owing to rapid clearance and poor tumour penetration, however a recently completed a phase II clinical trial against superficial bladder cancer achieved a 71% response rate. In this study, EO9 was administered intravesically and its poor pharmacokinetic (PK) profile ensured that the compound was cleared in the urine without any detection in blood plasma. EO9 will enter phase III clinical trial in 2006[3]. Efforts to improve the PK profile of EO9 whilst maintaining the efficient bioactivation have led to the identification of (**15**) which has a plasma half-life ($t_{1/2}$) in mouse of 16 minutes compared to a $t_{1/2}$ of 2 minutes for EO9 [39].

RH1

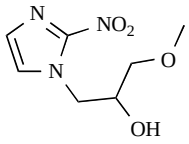
The quinone RH1 (**4**) is being developed as a bioreductive drug targeting tumours expressing high levels of DTD and is currently undergoing phase I/II clinical trials[4]. Activation is mediated by a two-electron reduction in tumour cells (aerobic and hypoxic) expressing high levels of DTD [40] whilst in tumour cells expressing low levels of DTD, RH1 is reduced by a one-electron mechanism resulting in the expected hypoxic selectivity [41]. Interestingly, profiling of RH1 against tumour cell lines (selected from the NCI's 60 tumour cell line panel) expressing various levels of DTD indicate that cytotoxicity was independent of DTD expression, suggesting the possibility that additional mechanisms may be involved in its activation [42].

NLCQ-1

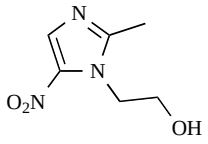
Much of the work on bioreductive compounds stemmed from the nitroimidazole radiosensitisers: misonidazole (**16)** and metronidazole (**17**) [43] (Fig. **7**). Both are metabolised under hypoxia to alkylating cytotoxins by a one-electron reduction mediated by the flavoprotein family of enzymes and is again governed by the *E(1)* potential of the nitro-group with the optimal range between -300mV and -450mV [44]. NLCQ-1 (**5**) was designed to have weak affinity for DNA in order to increase potency without adversely diminishing the distribution and diffusion to hypoxic regions [45]. The results have been successful and based on the encouraging data NLCQ-1 entered phase I clinical trials.

CB1954

Optimisation of Nitro-aniline mustards (Fig. **8**) led to the identification of **18** where the reduction of the nitro group under hypoxia leads to activation of the mustard [44]. Further development of this concept has identified the phosphate



16: Misonidazole



17: Metronidazole

Fig. (7).

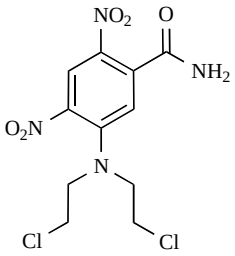
mustard PR-104 (**19**) that is more soluble and has a significantly better bystander effect. PR-104 been selected for clinical evaluation starting in December 2005[5]. Whilst, the related aziridine analogue, CB-1954 (**6**), has only a moderate hypoxic selectivity, it was found to be a good substrate for bacterial enzyme reductase (NTR) and is currently under clinical evaluation in combination with virus-directed enzyme prodrug therapy (VDEPT) [46]. As discussed earlier, a key desirable feature in hypoxia targeted therapies is the ability to kill neighbouring cells *via* a bystander effect. The activation of CB1954 by NTR generates a number of active metabolites, however the 2-amino metabolite CB10236 (**20**) is the key diffusible cytotoxin [47].

BIOREDUCTIVE PRODRUGS

The understanding of selective nitro reductions has led to the development of prodrugs which fragment selectively under hypoxia. This strategy has been used for preparing prodrug analogues of combretastatin (**21**) [48] and camptothecin (**22**) [49] (Fig. **9**). In a similar way, a recent publication details the potential of KS119 (**23**), a prodrug of the alkylating agent 90CE (**24**) as a hypoxic selective agent [50]. These prodrugs are reduced by an oxygen sensitive one-electron mechanism to unstable metabolites that fragment to generate the corresponding cytotoxin.

[4]Data presented at the Tumour–selective medicines conference organised by the Royal Pharmaceutical Society by Butler, J. on 12th Oct 2005.

[5]Advances in Chemical Approaches to Cancer Therapy, 25 Oct 2005, Denny, W.A. Clinical studies been initiated in NewZealand by Proacta Therapeutics Limited.



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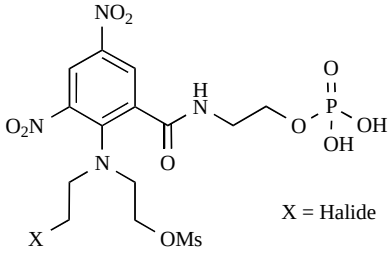
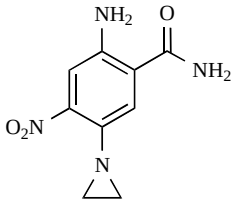
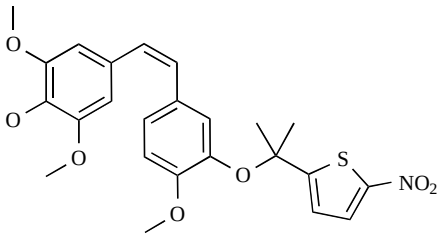


19: PR-104



20: CB10236

Fig. (8).



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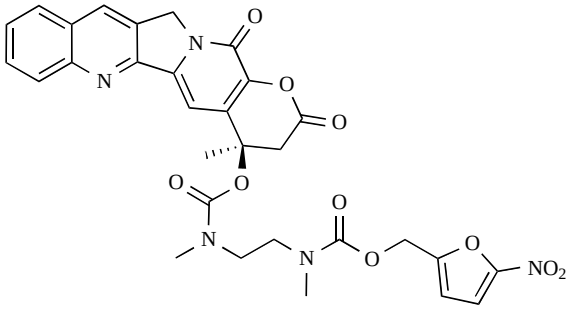

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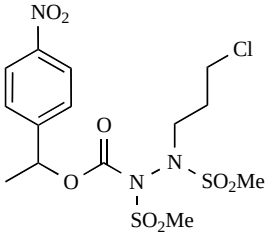
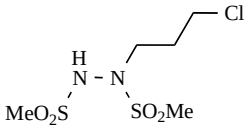


23: KS119



24: 90CE

Fig. (9).

CONCLUSIONS

The aim of this mini-review has been to outline the current clinical state of the art in hypoxia targeted chemotherapies. Hypoxia is a phenotype of Human solid tumours and given our understanding of the associated pathways and responses to this, targeting hypoxia is seen as a valuable approach for anti-cancer drug discovery. This review has focused on compounds, currently undergoing clinical trials, which target solid tumours and exploit hypoxia during the targeting event. Although the ultimate mechanism by which these compounds will kill a hypoxic tumour cell is varied, the ultimate aim of each is to deliver maximum levels of cytotoxin to the tumour whilst maintaining low systemic toxicity, and as these first examples progress through the clinic, we expect to gain a clearer insight into how this exciting branch of cancer therapy will progress.

ACKNOWLEDGEMENTS

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