Bioreductive agents

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The prognostic significance of 18F-misonidazole (FMISO) PET-detected tumor hypoxia in patients with advanced head and neck cancer randomly assigned to chemoradiation with and without tirapazamine

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Background: 45 patients were enrolled on a hypoxic imaging sub-study of a larger TROG trial in which patients with Stage III or IV SCC of the head and neck were randomized to radiotherapy (70Gy/35fx/7weeks) plus either cisplatin and infusional 5FU during weeks 6 and 7 (CIS/5FU) or the hypoxic cytotoxin, tirapazamine and cisplatin in weeks 1, 4 and 7 and tirapazamine alone in weeks 2 and 3 (CIS/TPZ) *(Proc ASCO 22: 495, 2003)*. The objective was to determine the association between tumor hypoxia and treatment regimen with local-regional failure (LRF) and failure-free survival (FFS).

Methods: Pretreatment FMISO PET was performed 2 hours after tracer administration with qualitative scoring of uptake in both primary tumours and nodes. Scores of 2 (mildly increased above background) or grade 3 (moderate-high) were deemed positive for the presence of hypoxia. FMISO scans were done at baseline and repeated in weeks 4–5 in patients with baseline hypoxia.

Results: 32 (71%) patients had detectable hypoxia in either or both primary and nodal disease. Hypoxia was present in the primary tumours of 17 of 45 (38%) patients and in the nodes of 21 of 38 (55%) 5 (60%) node positive patients. In patients who received the CIS/5FU regimen, 1/10 without any PET evidence of hypoxia had LRF compared to 8/13 of those with hypoxia: time to LRF was significantly shorter in hypoxic patients (exact logrank P=0.038; hazard ratio (HR)= 7.1). By contrast, in patients who received the CIS/TPZ regimen, only 1/19 patients with hypoxic tumours had LRF: time to LRF was significantly shorter in CIS/5FU patients (P=0.001; HR=15).

In patients who received the CIS/5FU regimen, 3/10 without any PET evidence of hypoxia had failed or died compared to 11/13 of those with hypoxia: FFS was shorter in hypoxic patients (exact logrank P=0.095; hazard ratio (HR)= 3.2). By contrast, in patients who received the CIS/TPZ regimen, only 5/19 patients with hypoxic tumours had failed or died: FFS was significantly shorter in CIS/5FU patients (P=0.004; HR=4.7).

2, 9, 8/32 patients with baseline hypoxia had a repeat FMISO scan in weeks 4 or 5. 4/12 on CIS/5FU had residual hypoxia and all had LRF, while 2/16 on CIS/TPZ had residual hypoxia with neither experiencing LRF.

Conclusions: Hypoxic PET imaging can containing chemoradiation regimen. The decreased LRF rate in patients with hypoxic tumors who received CIS/TPZ supports the pre-clinical data that tirapazamine acts by specifically targeting hypoxic tumour cells. Baseline hypoxia and persistent hypoxia in patients receiving the non-tirapazamine-containing regimen are are associated with a high risk of local-regional failure.

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Novel hypoxia-selective 1,2,4-benzotriazine 1,4-dioxides with improved extravascular transport: analogues of Tirapazamine with in vivo activity

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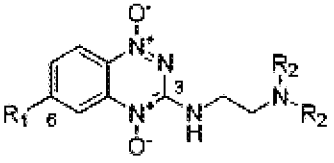
Tirapazamine (TPZ) is a bioreductive hypoxia-selective cytotoxin currently in Phase III clinical trial in combination with chemo-radiotherapy. Despite encouraging indications of clinical activity, TPZ shows considerable toxicity which precludes delivery of the target dose in a high proportion of patients. There is also considerable evidence that extravascular transport (EVT) limits the antitumour activity of TPZ. In particular, multicellular layer (MCL) culture models have shown that metabolic consumption of TPZ is fast enough to limit its penetration into hypoxic tissue.

We have designed a series of 1,2,4-benzotriazine 1,4-dioxide (BTO) analogues in an effort to optimize EVT by increasing tumour tissue diffusion through increased lipophilicity, and decreasing metabolism by lowered electron affinity, whilst improving aqueous solubility and maintaining hypoxic selectivity. The addition of 3-NH-alkylamino sidechains was designed to increase solubility and electron-donating 6-substituents (R_1) were used to balance the effect of the 3-substituents on the electron affinity of the BTO nucleus. Lipophilic amines (NR_2R_2) of moderate pKa and lipophilic R_1 groups were chosen to improve the diffusion of the BTOs. The analogues show excellent solubility compared to TPZ. The one-electron reduction potentials, determined by pulse radiolysis, range from −396 to −555 mV (TPZ, −456). In vitro hypoxic cytotoxicities (IC_{50} values) were determined in human colon carcinoma HT-29 cells and range from 0.58 to 36.5 μM (TPZ, 4.9), while hypoxic selectivities (HCR = aerobic IC50/hypoxic IC50) range from 15 to 215 (TPZ 67). Diffusion coefficients in HT-29 MCLs (D_{MCL}) range from 1.87 to 17.1×10^{-7} cm^2 s^{-1} (TPZ, 3.97) and correlate with $logP_{7.4}$. First order rate constants for hypoxic metabolism (k_{met}), estimated at the C_{10} (concentration for one log of cell kill after 1 h of drug exposure in cell suspensions), range from 0.15 to 9.16 min^{-1} (TPZ, 0.60). The calculation of a one-dimensional transport parameter (X_*, penetration distance into hypoxic tissue) allows comparison of EVT among BTOs and identifies analogues with improved EVT compared to TPZ. In vivo evaluation revealed several analogues that demonstrate significant killing of hypoxic cells in HT-29 xenografts



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Exploiting nitric oxide synthase (NOSII) overexpression in hypoxic tumour cells to improve response to the bioreductive drug AQ4N

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AQ4N is a novel di-*N*-oxide prodrug topoisomerase II inhibitor that can undergo two sequential 2-electron reductions via AQ4M (mono-*N*-oxide) to a four-electron stable oxygen-insensitive DNA-affinic topo-II inhibitor AQ4. We have recently demonstrated that up-regulation of nitric oxide synthase (NOSII) in solid tumours could provide tumour selective metabolism of bioreductive drugs [CHINJE et al. *Mol Pharmacol* 63:1248–1255, 2003]. NOS consist of an oxidase and reductase domain and a number of studies to date have revealed a requirement for either cytochrome P450 or the presence of both heme and P450 reductase (P450R) in the reductive metabolism of AQ4N. This observation raised the possibility that, NOS by the nature of its dimeric heme-reductase domains, may also support metabolism of AQ4N.

In this study an expression vector containing the cDNA for human inducible NOS (h-iNOS) was utilized to transfect HT1080 fibrosarcoma cells. A clone was selected (HT-NOS12) that stably over-expressed NOSII activity as determined by the conversion of ^{14}C radiolabelled L-arginine to citrulline (95 pmol/min/mg compared to 0.89 pmol/min/mg for parental cells). Catalytic activity of the reductase domain as determined by the NADPH-dependent reduction of cytochrome c was also elevated (24nmol/min/mg for HT-NOS12 compared to 5.2 nmol/min/mg for HT1080wt). Hypoxic incubation for hplc analysis was performed under zero-grade nitrogen passed through an oxy-trap and metabolite detection was at 242nm.

NADPH-dependent metabolism of AQ4N determined by HPLC analysis was supported by this increased NOS expression predominantly to the 2-electron reduced product, AQ4M (131 pmol/min/mg for HT-NOS12 compared to 7 pmol/min/mg for HT1080wt), representing a 19-fold increase. The cytotoxic metabolite AQ4 was not detectable in HT1080wt but this was elevated to 10.3 pmol/min/mg in the HT-NOS12 clone. Additionally we have shown that at intermediate oxygen levels (0.1–2%), characteristic of hypoxic tumours, elevated NOSII expression significantly sensitises these tumour cells to radiation treatment. For example at 0.5% O_2 nitric oxide sensitization enhancement ratio (NO-SER) was significantly greater for HT-NOS12 clone (2.2) compared to 1.5 for HT1080wt. HT-NOS12 clones also formed xenografts in cba *nu/nu* mice *in vivo* that maintained elevated NOSII expression. *S*-9 fractions derived from these xenografts metabolised AQ4N predominantly to AQ4M (94 pmol/min/mg for HT-NOS12 compared to 5.4 pmol/min/mg for HT1080wt). However, AQ4 metabolite formation could only be detected when hypoxic incubation was carried out with HT-NOS12 clone (19 pmol/min/mg). Further evaluation of this tumour model for AQ4N drug response when administered alone or in combination with radiotherapy is currently in progress.

In conclusion, we have demonstrated that elevated NOSII activity in hypoxic tumours may present a unique opportunity for targeting radio- and chemo-resistant tumours with AQ4N currently under clinical investigation.