BIO-REDUCTIVE METABOLISM OF SMALL MOLECULE NITROAROMATICS AND N-OXIDES IN HYPOXIA

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ABSTRACT

Hypoxia in tumors causes adverse effects to therapy and negatively impacts on patient prognosis. Identification and quantification of hypoxia is considered to have a strong impact on treatments in tumor therapy. Fluorescent-based detection to mark hypoxia may be vital to be used along with available methods such as radiochemical and immunohistochemical staining.

In this work, the non-fluorescent 6-nitroquinoline (**42**) was used to investigate the production of a fluorescent 6-aminoquinoline (**43**) and other metabolites under bio-reducing hypoxic conditions. In the presence of the enzymatic reducing system NADPH:cytochrome P450 reductase/NADPH, 6-nitroquinoline (**42**) produced the fluorescent helicene (**44**), along with the non-florescent azo (**45**). An authentic sample of (**44**) was chemically synthesized and characterized and used to confirm the production of this molecule in the enzymatic process. Interestingly, the expected fluorophore (**43)** is not produced by NADPH:cytochrome P450 reductase/NADPH.

In another study, the enzymatic reducing system xanthine/xanthine oxidase was used to reduce (**42**) under hypoxia to obtain (**43**). In these experiments (**43)** was produced and the yield is increased with xanthine concentration. Metabolic identification revealed that intermediates of typical nitro reduction pathway are present along with 6-nitroquinolone (**51**).which is formed by xanthine oxidase mediated oxidation of (**42**). The absence of (**44**) as a metabolite with xanthine/xanthine oxidase system highlights the complexity of bio-reduction of nitroaromatics under hypoxia.

In our laboratory, bio-activation of di-*N*-oxides such as tirapazamine (TPZ, **42**) has been studied. TPZ undergoes one-electron bio-reduction to produce oxidizing radical, which causes

DNA damage under hypoxia. In our laboratory, the mechanism by which TPZ mediated DNA damage has been investigated using TPZ and its analogs.

Our evidence suggests that upon undergoing bio-reduction, TPZ produces hydroxyl radical as the DNA damaging radical species. Others have suggested another mechanism, which proposes the formation benzotriazine radical (**38**) upon dehydration process over the bio-reduction step. In the current work, TPZ analog 1,2,4-benzotraizine-1,4-dioxide (**55**) and deuterated (**60**) were used to test the dehydration mechanism. Isotopic content analysis of metabolites, derived from bio-reducing metabolism of (**55**) and its deuterated analog (**60**), using HRMS show evidence against the dehydration mechanism.