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Comparison of the nucleic acid covalent binding capacity of two nitro-substituted benzazolo[3,2-*a*]quinolinium salts upon enzymatic reduction

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Abstract

The DNA binding capacity of two nitro-substituted benzazolo[3,2-*a*]quinolinium chlorides (NBQs), NBQ-38 and NBQ-95, was evaluated upon their enzymatic reduction with hypoxanthine (HX)/xanthine oxidase (XO) under anaerobic conditions. In the presence of 2′-deoxyguanosine (2′-dG) or calf thymus DNA, covalent-addition products were monitored. Reactions of each NBQ with 2′-dG or DNA differed in the NBQ to HX molar ratio. Control reactions, one without HX/OX and another under aerobic conditions, were also analyzed. Adducts were isolated and characterized by high performance liquid chromatography (HPLC) and electrospray ionization-mass spectrometry (ESI-MS). Authentic samples of the reduced forms of these NBQs, identified as ABQ-38 and ABQ-95, were synthesized as standards to monitor bioreduction processes. HPLC analysis showed that the yield of formation of an unknown product (possibly, 2′-dG-NHBQ-38 adduct) from the reaction of NBQ-38 with 2′-dG and DNA was proportional to the HX to NBQ-38 molar ratio. ESI-MS analysis of the DNA hydrolysates showed evidence of an adduct formed upon bioreduction of NBQ-38 by the ions detection at *m/z* 528.3 and 454.8, consistent with chemical structures of a 2′-dG-NHBQ-38 adduct and a fragment ion. DNA adducts were not observed with NBQ-95, although the corresponding bioreduction product ABQ-95 was detected by ESI-MS. This study provides mechanistic information of these bioreductively-activated pro-drugs with potential therapeutic applications.

Keywords: DNA adducts; Bioreductive drugs; Benzazolo[3,2-*a*]quinolinium; Hypoxia; Mass spectrometry

1. Introduction

Hypoxic environments in solid tumors continue to challenge conventional treatment. Many bioreductive drugs cited in the literature are presumed to be activated when the drug or a drug component (effector) is reduced (Laderoute et al., 1988; Denny et al., 1996). Furthermore, an enhanced production of hypoxanthine (HX) together with the proteolytic conversion of xanthine deoxydehydrogenase to xanthine oxidase (XO) during ischemia have been

Abbreviations: BQ, benzazolo[3,2-*a*]quinolinium chloride; NBQ or BQ-NO_2, nitrobenzazolo[3,2-*a*]quinolinium chloride; ABQ or BQ-NH_2, aminobenzazolo[3,2-*a*]quinolinium chloride; NBQ-38, 7-ethyl-3-nitrobenzimidazo[3,2-*a*]quinolinium chloride; NBQ-95, 10-methyl-2-chloro-3-nitrobenzothiazolo[3,2-*a*]quinolinium chloride; ABQ-38, 3-amino-7-ethylbenzimidazolo[3,2-*a*]quinolinium chloride; ABQ-95, 3-amino-2-chloro-10-methylbenzothiazolo [3,2-*a*]quinolinium chloride; 2′-dG, 2′-deoxyguanosine; 2′-dG-NHBQ-38, adduct formed in the bio-reduction of NBQ-38 in the presence of 2′dG or DNA; HX, hypoxanthine; XO, xanthine oxidase; ESI-MS, electrospray ionization mass spectrometry; CID, collision-induced dissociation.

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implicated in the generation or initiation of a hypoxic and chemically reducing environment (Wardman, 2001).

Recent interest in nitro compounds has focused on therapeutic applications; in particular, their selectivity in the treatment of hypoxic solid tumors (Price et al., 2000; Borch et al., 2001). Hypoxic solid tumors can show strong resistance to traditional therapeutic approaches such as radiotherapy. Drugs activated in hypoxia by reductase enzymes have the potential, however, to overcome this resistance to radiotherapy. Bioreductive-activated drugs such as nitroarenes, quinones, or those drugs with aromatic *N*-oxide moieties have been known to possess redox properties and to present selective toxicity toward cells with limited oxygen content, such as those within a hypoxic, solid tumor (Wardman, 2001).

The reason for the selectivity of the nitroarene compounds is that in hypoxic tissues, reduction of the nitro group by intracellular metabolic reductases can result in strong electrophilic intermediates and DNA-binding species, i.e. hydroxylamine derivatives. In normoxic tissues, the reduced derivatives could be reoxidized very efficiently by oxygen, thus, inhibiting the formation of DNA-alkylating species (Adams, 1992). Intermediates derived from the bio-oxidation of aromatic amines are known to form covalent DNA adducts in which binding occurs preferentially at the C8 position of the guanine bases. Drugs that interact or bind to DNA could affect cellular processes such as transcription or DNA replication, initiating cell death mechanisms such as apoptosis (Mello et al., 1995).

NBQ-38 (7-ethyl-3-nitrobenzimidazo[3,2-*a*]quinolinium chloride) and NBQ-95 (10-methyl-2-chloro-3-nitrobenzothiazolo[3,2-*a*]quinolinium chloride) (Fig. 1 and Table 1) are nitro-containing heterocyclic compounds possessing a positive charge that could facilitate their interaction with cellular organelles. They belong to a new family of unnatural alkaloids known as benzazolo[3,2-*a*]quinolinium salts (BQ). Of these, 3-nitrobenzothiazolo[3,2-*a*]quinolinium chloride (NBQ-2) has shown potential as an anticancer agent (Cox et al., 1982). It has been shown that these planar heterocyclic cationic nitroarenes can be reduced electrochemically or bioreduced in the presence of HX/XO (Cox et al., 1999; Alegria et al., 1993, 2004). However, nothing is known regarding the possibility of DNA alkylation mediated through nitro reduction by this family of

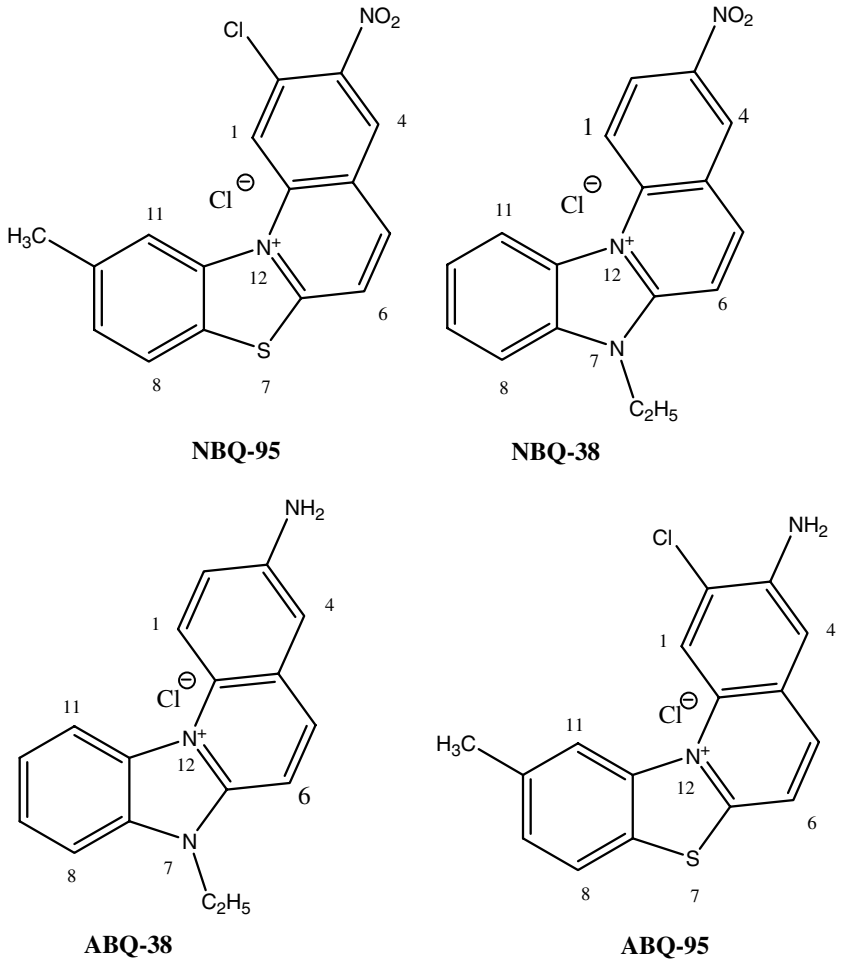

Fig. 1. Structure of NBQs (NBQ-38 and NBQ-95) studied and their corresponding amino derivatives, ABQs, (ABQ-38 and NBQ-95) used to monitor the bio-reduction process.