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Guanine Nucleotide Oxidation and Cell Death by Bactericidal Antibiotics

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ABSTRACT

Emergence of antibiotic resistance by pathogenic bacteria is a very serious problem nowadays. So for the derivation of new class of antibiotics and clinically useful adjuvants for current antimicrobial therapies, a detailed understanding of the mechanisms underlying the antibiotic killing is important. Its shown that the three major classes of bactericidal antibiotics (β-lactams, quinolones and aminoglycosides), regardless of drug target interaction, stimulate the production of highly deleterious hydroxyl radical in gram negative and gram positive bacteria. These hydroxyl radicals ultimately cause cell death by specific oxidation of guanine nucleotide pool and its subsequent use in nucleic acid transactions by a tranlesion DNA polymerase called Din B polymerase that can use 8-oxo-deoxyguanosine triphosphate (8-oxo-dGTP) as the incoming nucleotide pairing it with either deoxycytidine or deoxyadenosine (dC or dA), with a preference to dA. This will lead to accumulation of lethal mutations or formation of lethal double stranded breaks (DSB) resulting in cell death.

KEYWORDS : 8-oxoguanine, Hydroxyl radicals, Din B polymerase.

Introduction

A detailed understanding of the mechanisms that underlie antibiotic killing is important for the derivation of new classes of antibiotics and clinically useful adjuvants for current antimicrobial therapies. Antibiotics like Beta-lactams, aminoglycosides and quinolones targets different macromolecules for their bactericidal effect. These antibiotics increase intracellular levels of OH free radical and cause damage to DNA, lipids, proteins and generalized oxidation catastrophe could result in cell death (Kohanski et al., 2007). However, recent studies suggest that cell death is predominantly elicited by specific oxidation of the guanine nucleotide pool and its subsequent use in nucleic acid transactions. These studies proved that cytotoxicity of beta-lactams and quinolones predominantly results from lethal double-strand DNA breaks caused by incomplete repair of closely spaced 8-oxo-deoxyguanosine lesions, whereas the cytotoxicity of aminoglycosides might additionally result from mistranslation due to the incorporation of 8-oxo-guanine into newly synthesized RNAs.

Mechanism Of Acton Of Beta-Lactams, Aminoglycoside and Quinolones

Beta-lactams interact with penicillin binding protein and glycopeptides that interact with peptidoglycan building blocks and interfere with normal cell wall synthesis and induce cell lysis and cell death. (Reynolds, 1989). Aminoglycoside binds to the ribosome subunits and cause protein mistranslation (Davis, 1987; Weisblum and Davies, 1968). Quinolones target DNA replication and repair by binding DNA gyrase complexed with DNA, which drives double stranded break formation and cell death (Drlica and Zhao, 1997).

ROS and cell death

Incomplete transfer of electrons to O_2 during oxidative phosphorylation yields products such as hydrogen peroxide, hydroxyl radicals and singlet oxygen species, which jointly comprise ROS. Among this hydroxyl radical formation utilizing internal iron in the Fenton reaction appears to be the most significant contributor to cell death among the reactive oxygen species formed. (Imlay et al., 1988; Imlay and Linn, 1986). The Fenton reaction leads to the formation of hydroxyl radicals through the reduction of hydrogen peroxide by ferrous iron.

Bactericidal antibiotics and ROS

Bactericidal Antibiotics also Induce Hydroxyl Radical Formation. Hydroxyl Radical Formation for All Bactericidal Classes Involves the Fenton Reaction and utilizes Intracellular Iron. Catabolic NADH Depletion Is the Trigger for Hydroxyl Radical Formation (Kohanski et al., 2007). Bacterial gyrase inhibitors, including synthetic quinolone antibiotics and the cytotoxic protein CcdB, induce a breakdown in iron regulatory dynamics, which promotes formation of reactive oxygen species that contribute to cell death (Dwyer et al., 2007). But recent works suggest that cell death is predominantly elicited by specific oxidation of the guanine nucleotide pool and its subsequent use in nucleic acid transactions (James et al., 2012). It is also reported that amongst the four normal nucleotide bases, guanine is the most susceptible to oxidation due to its low oxidation potential (Steenken and Jovanovic,1997).

Formation of 8-oxoGuanine

The formation of 8-oxoGua in DNA has been shown to occur via two pathways. It can be either directly by oxidation of Guanine in DNA or indirectly via oxidation of dGTP in the nucleotide pool to 8-oxodGTP, followed by incorporation of 8-oxodGTP into the DNA by DNA polymerase(s) (Sekiguchi and Tsuzuki, 2002).

DinB POLYMERASE and Cell Death

DinB Polymerase otherwise called as DNA Polymerase IV is an E coli translation DNA polemerase. It can use 8-oxo d GTP as the incoming nucleotide, pairing with either dC or dA (preference to dA). A bond between the Arginine residue at 332 position of the enzyme with 8-oxodG playes a role in determining the fidility and efficiency of Din B catalyzed bypass. The elevated levels of DinB were lethal because of the increased use of oxidized deoxynucleotides, rather than because of the induction of high levels of OH radical. DinB overproduction causes cell death by incorporating more 8-oxo-dG than the cell can tolerate. Bactericidal antibiotic lethality, in part, is due to the oxidation of guanine nucleotides and thereby increasing the number of DSBs (James et al., 2012).

Conclusion

Bactericidal antibiotics will increase the intracellular OH free radicals through fentons reaction utilizing intracellular iron. These free radicals will cause the formation of 8-oxodG either by directly oxidising guanine in DNA or indirectly by oxidising dGTP and further incorporating into DNA by DNA polymerase(s). 8-oxodG pairs with either dC or dA (preference to dA) which induced lethal mutation to the cell. A bacterial translation DNA polemerase called DinB incorporates more 8-oxodG than the cell can tolerate and causes cell death.

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