https://doi.org/10.70200/RX202401041J

NOVEL TARGETED VIOLOGEN FOR THE INDUCTION OF SUPEROXIDE PRODUCTION IN MITOCHONDRIA

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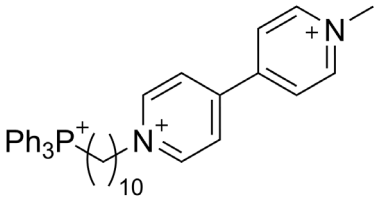
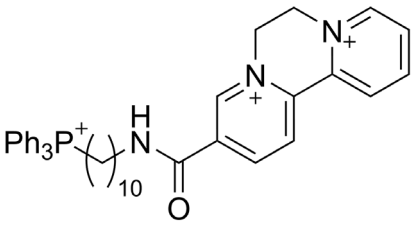
Mitochondrial production of $O_2^{\bullet-}$ and H_2O_2 has been implicated in redox signaling and in the pathogenesis of numerous diseases including cancer, neurodegeneration, and cardiovascular diseases. To understand the exact role of those species, new chemical biology tools for selective and efficient induction of mitochondrial superoxide production are needed. Here, we report the development of a new viologen-based redox cycling agent, mito-diquat (Mito-DQ), capable of inducing targeted mitochondrial $O_2^{\bullet-}$ production at significantly higher rates as compared to previously reported mito-paraquat (Mito-PQ), a widely used chemical tool to study mitochondria-dependent redox signaling[1,2].



Mito-PQ



Mito-DQ

Mito-DQ was synthesized by coupling a diquat (DQ) moiety to a mitochondria-targeting triphenylphosphonium cationic group via an alkyl linker[3]. To study the redox cycling activity of Mito-DQ in a cell-free system, xanthine oxidase (XO)-catalyzed oxidation of NADH as well as isolated bovine heart mitochondria were used as sources of $O_2^{\bullet-}$. Pulse radiolysis experiments were performed to characterize the radical species produced upon one-electron reduction of Mito-DQ and to determine the rate constant of its reaction with molecular oxygen to produce $O_2^{\bullet-}$. Induction of oxidant production in intact cells was studied using C2C12 myoblasts. Cellular production of $O_2^{\bullet-}$ was measured using high-performance liquid chromatography (HPLC) whereby hydroethidine probe oxidation to 2-hydroxyethidium was monitored. Stimulation of H_2O_2 production was measured by determining the rate of catalase-sensitive conversion of Amplex Red to resorufin, catalyzed by horseradish peroxidase. Our results indicate that Mito-DQ stimulates NADH oxidation, O_2 consumption, and $O_2^{\bullet-}$ production by NADH/XO system

in a dose-dependent manner (0.1-100 μM) and in isolated mitochondria. Mito-DQ-derived radical is stable in the absence of molecular oxygen, while decays within 200 μs in an air-equilibrated solution. Mito-DQ dose-dependently (1-100 μM) induced $O_2^{\bullet-}$ and H_2O_2 production in C2C12 cells under the conditions when no significant stimulation of oxidant production is observed for Mito-PQ. We conclude that Mito-DQ may be a useful chemical tool to study the role of mitochondrial $O_2^{\bullet-}$ production in model biological systems.

References:

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