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Selective induction of oxidative stress in cancer cells via synergistic combinations of agents targeting redox homeostasis

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ABSTRACT

Cancer cell resistance to chemotherapy is still a heavy burden that impairs the response of many cancer patients to conventional chemotherapy. Using drug combinations is one therapeutic approach to overcome the developing resistance to any one drug. Oxidative stress is now a generally regarded hallmark of cancer that can be one approach to selectively target cancer cells while sparing normal cells. With the aim of increasing oxidative stress in cancer cells to a lethal set point, we have generated and combined several series of redox active compounds that act at different points of the cellular oxidative cascade. The premise of such combinations is to deplete of endogenous antioxidant defence proteins (e.g., Glutathione) while concomitantly increasing the generation of ROS via metal redox recycling and Fenton chemistry which eventually leads to the disruption of cellular redox homeostasis and induction of cell death. Through this approach, we have identified highly synergistic combinations of two distinctive classes of compounds (Azines and Copper(II) complexes of 2-pyridyl ketone thiosemicarbazones) which are capable of eliminating cancer cells without concomitant increase in toxicity toward normal cells. In one of our most potent combinations, a combination index (*CI*) value of 0.056 was observed, representing a 17 fold enhancement in activity beyond additive effects. Such new combination regimen of redox active compounds can be one step closer to potentially safer low dose chemotherapy.

1. Introduction

Breast cancer is the most commonly diagnosed cancer among females in the Asia-Pacific region, representing about 18% of all cancer cases in the region in 2012.[1] It is also the fourth leading cause of cancer-related deaths among females in the region. Despite the availability of new chemotherapeutic agents, the overall mortality trends are still rising in several countries; rendering it necessary to develop new agents with higher potency and selectivity profile in order to ensure the best treatment outcome.[1]

Cancer cells operate under substantially higher level of intrinsic oxidative stress than normal cells (Fig. 1), which is attributed to oncogenic transformation, metabolic reprogramming and subsequent increase in reactive oxygen species (ROS) generation.[2] Because of such heightened basal level of ROS, cancer cells are more susceptible to an increase in oxidative stress through a drug pro-oxidant intervention that augments ROS levels or weakens the antioxidant defences (which reduce intrinsic oxidative stress) in cancer cells shifting the set point toward the threshold line to cause cell death.[3] A similar increase in ROS levels would not be sufficient to elicit this effect in a normal cell.

Under normal physiological conditions, cellular redox homeostasis is controlled by three systems; the reduced glutathione (GSH)/oxidised glutathione (GSSG); the glutaredoxin (Grx) system; and the thioredoxin (Trx) system.[6] GSH, Grx and Trx; each modulate the cellular redox status by trapping ROS, or by reversing the formation of disulfide oxidation products (RSSR).[6a] Those systems are dependent directly or indirectly on NAD(P)H to regenerate the reduced active form (RSH) via a flavin oxidoreductase enzyme.[7] When ROS levels inside a cell build above the tolerance threshold, cell death is usually triggered via oxidative damage of cellular components.[8] DNA is one of the primary targets to the oxidative damage of ROS (Fig. 2).[9]

Flavin oxidoreductase is one of the targets of anticancer oxidative therapy.[10] Azines are flavin analogues that can act as competitor of flavin cofactors in the hydride transfer to RSH proteins (Fig. 2) that are responsible for the maintenance of cellular homoeostasis.[11] Different classes of compounds are categorised under the term 'Azine' have demonstrated potential anticancer activity; riminophenazines [e.g., clofazimine (CFZ), Fig. 3],[12] phenothiazines [e.g., methylene blue (MB) Fig. 3][13] and phenoxazines (e.g., benzo(a)phenoxazine, Fig. 3).[14] Each of those classes has an

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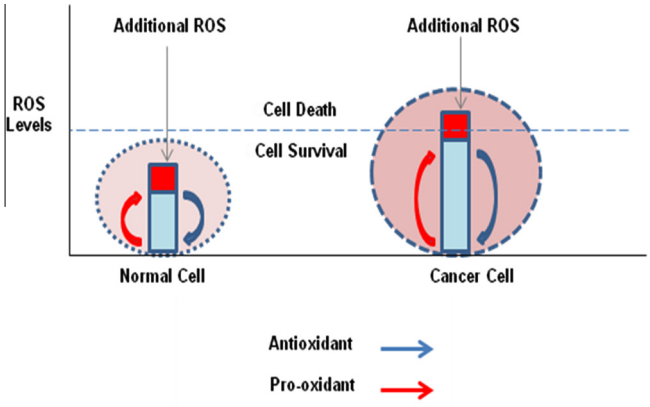


Figure 1. Biological difference between a normal cell and a cancer cell in terms of intrinsic oxidative stress which provides basis for therapeutic selectivity of oxidative therapy against cancer cells. Adapted from Gupte et al. (2009)[4] and Trachootham et al. (2009).[5]

active oxidised pharmacophore within its structural core and 2-electron reduction can proceed at biologically relevant potentials.[15]

An emerging target in anticancer oxidative therapy is intracellular generation of ROS via metal chelators.[16] One of the early metal chelators was the thiosemicarbazone 3-AP (Fig. 3) which was successfully used as anticancer in phase II clinical trials.[17] Thiosemicarbazones can chelate both intracellular iron and copper, among other metals.[18] The mechanism by which they act is dependent not only on metal chelation but also on metal redox cycling of their metal complexes leading to formation of ROS.[19] In their reduced forms (Fe^{2+} or Cu^{+}), they react with H_2O_2 in a Fenton like reaction generating hydroxyl radicals.[19] One of the most active thiosemicarbazone derivatives of di-2-pyridyl ketone is 4,4-dimethyl-3-thiosemicarbazone derivative (Dp44mT, Fig. 3).[20] Dp44mT has been demonstrated to reduce the growth of multiple tumors in vivo and in vitro in a more potent and less toxic fashion than 3-AP.[21]

Our premise is that induction of oxidative stress inside cancer cells using binary combinations of agents known to induce oxidative stress at different points of cellular oxidative cascade will result in enhanced cytotoxicity and faster elimination of cancer cells. The combination of a flavin analogue to deplete antioxidant thiol proteins and a metal chelator/metal complex to participate in intracellular Fenton's chemistry, is proposed to magnify intracellular generation of ROS while creating multiple futile consumption cycles

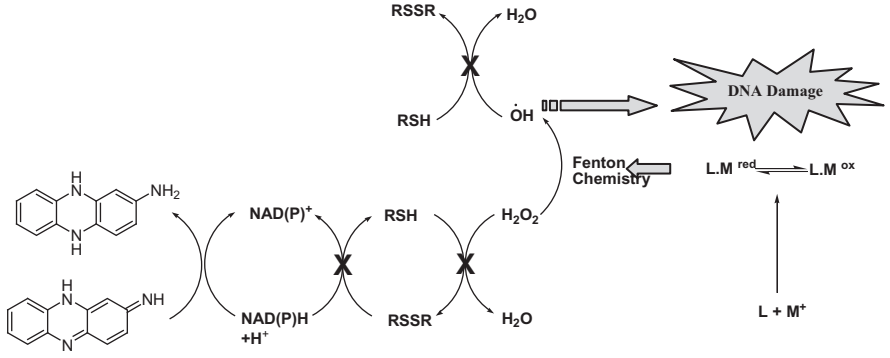
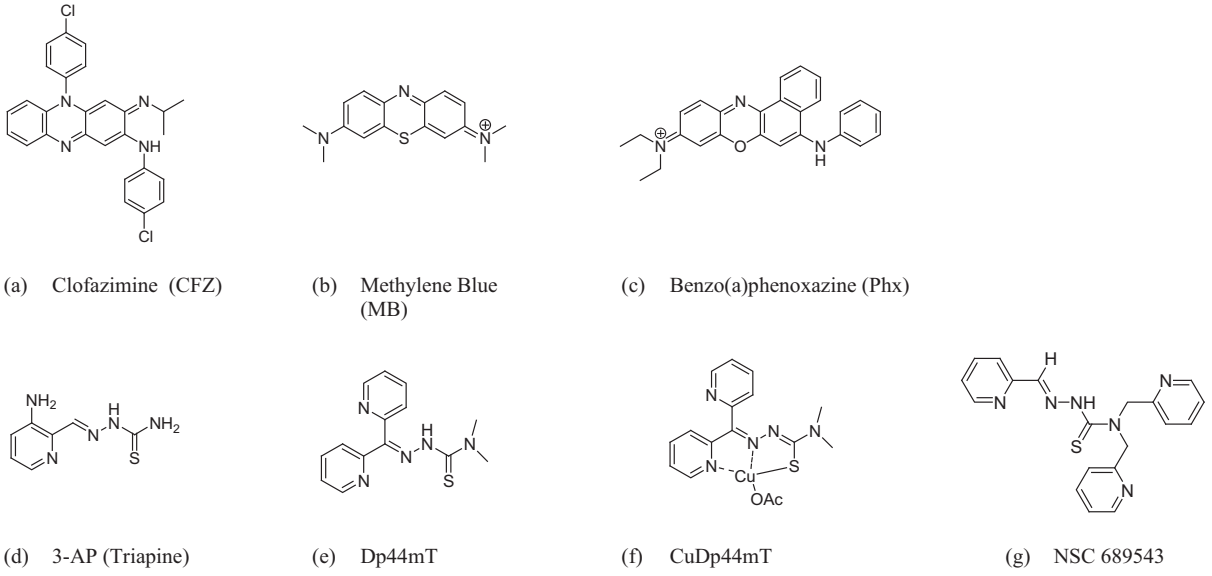


Figure 2. The impact of NAD(P)H depletion caused by an Azine (flavin analogue) and the increase in the generation of ROS caused by metal–chelator complex ($L.M^{+}$) via Fenton chemistry, on cellular redox homeostasis and subsequent accumulation of hydroxyl radicals inside the cell leading to DNA damage and cell death. NADP: Nicotinamide Adenine Dinucleotide Phosphate; RSH: reduced form of thiol proteins; RSSR: oxidised form of thiol proteins; L: thiosemicarbazone chelator; M^{+}: Cu or Fe, $L.M^{ox}$: oxidised metal–chelator complex, $L.M^{red}$: reduced metal–chelator complex.



(a) Clofazimine (CFZ) (b) Methylene Blue (MB) (c) Benzo(a)phenoxazine (Phx)

(d) 3-AP (Triapine) (e) Dp44mT (f) CuDp44mT (g) NSC 689543

Figure 3. Examples of redox active compounds (a–f) generating ROS inside cells; CFZ (a), MB (b) and Phx (c) are flavin analogues. 3AP (d), Dp44mT (e) and NSC 689543 (g) are metal chelators, CuDp44mT (f) is the Copper(II) complex of Dp44mT ligand.

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