

Tumor-Specific Induction of Oxidative Stress with Dichloroacetate and Auranofin May Complement the Efficacy of PARP Inhibition in Cancer Control

Mark F. Mc Carty

Catalytic Longevity, 7831 Rush Rose Dr., Apt. 316, Carlsbad, CA 92009, USA

*Corresponding author: Mark F. Mc Carty, Catalytic Longevity, 7831 Rush Rose Dr. Apt. 316, Carlsbad, CA 92009, USA ; Tel: 760-216-7272; Fax: 760-704-6379; E-mail: markfmcarty@gmail.com

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Abstract

PARP inhibition can improve survival in cancer patients whose tumors have impaired capacity for homologous recombination, such as germ line or somatic mutations in BRCA. The efficacy of such therapy should be greater if the rate at which cancer cell DNA incurs single-strand breaks is enhanced. Since oxidative stress promotes formation of such breaks, measure which boost oxidative stress in cancer – preferable somewhat selectively – may be worthwhile adjuvants to PARP therapy. In the many cancers that express the Warburg phenomenon, dichloroacetate can promote increased mitochondrial generation of oxidants by directing more pyruvate to oxidation in the Krebs cycle. Concurrent administration of the arthritis drug auranofin could further enhance mitochondrial release of hydrogen peroxide by indirectly inhibiting peroxiredoxin-3, the chief mitochondrial source of peroxidase activity. The copper-chelating drug tetrathiomolybdate, employed in cancer therapy as an anti-angiogenic agent, can boost superoxide levels by diminishing activity of the copper-zinc-dependent cytosolic superoxide dismutase. Episodic intravenous infusion of high-dose ascorbate could also be employed to increase oxidative stress in the cancer and thereby complement PARP inhibitor therapy. The potential of dichloroacetate, auranofin, tetrathiomolybdate, and intravenous ascorbate to enhance the cancer-retardant efficacy of olaparib (or other PARP inhibitors) could be assessed in cell culture and rodent models.

Keywords: PARP; Olaparib; Oxidativestress; Dichloroacetate; Auranofin; Tetrathiomolybdate; Ascorbate

Cancer Therapeutic Potential of PARP Inhibition

PARP inhibitors are potentially useful for the control of cancers with impaired capacity for repair of double-strand breaks by homologous recombination (HR) [1]. These include cancers carrying genomic or somatic mutations of BRCA, or that for other reasons lack efficient HR. A key determinant of the success of PARP inhibitor therapy will be the rate at which single-strand DNA breaks are formed in cancer cells. By impeding the base excision repair mechanism required for remediation of single strand breaks, PARP inhibition increases the chance that double strand breaks will occur, most notably during S phase DNA replication. Capacity to repair such double strand breaks will be impaired in cells with inefficient HR, and unrepaired double strand breaks can induce apoptosis via p53 signaling or other mechanisms.

Boosting Oxidative Stress in Cancers

Oxidative or nitroxidative stress in cancer cells is capable of inducing single strand breaks, and many cancers are in a state of mild oxidative stress [2-4]. Measures which somewhat selectively up-regulate this oxidative stress in cancer cells can therefore be expected to potentiate the efficacy of PARP inhibitory therapy. In cancers that express an active Warburg phenomenon-reflecting constitutive activation of HIF-1 that drives glycolysis and inhibits mitochondrial oxidation of pyruvate by boosting pyruvate dehydrogenase kinase (PDK) expression – the PDK inhibitor dichloroacetate (DCA) boosts mitochondrial respiration and concomitant production of superoxide

[5-7]. This increase in oxidative stress will tend to be cancer-specific, owing to higher PDK activity in many cancers, as well as the fact that cancer mitochondria tend to have dysfunctional respiratory chains, reflecting the genetic lability of cancer [8-10].

Mitochondrial release of hydrogen peroxide can be further boosted by inhibitors of thioredoxin reductase (TR), as thioredoxin-2 is the source of reductive power for peroxiredoxin-3, an antioxidant enzyme which contributes importantly to hydrogen peroxide disposal in mitochondria [11-13]. Peroxiredoxin-3 is c-Myc-inducible, and many cancers show increased expression of this enzyme [14-16]. The venerable anti-arthritic gold drug auranofin inhibits thioredoxin reductase potently, with a K_i of only 4 nM, reflecting an interaction between gold and the selenocysteine in its active site [17,18]. When added to cell cultures, 0.3 μ M auranofin achieves a 50% inhibition of the mitochondrial fraction of TR [19]. The standard clinical regimen of this drug – usually 3 mg twice daily – may be sufficient to achieve partial inhibition of TR activity systemically.

Further Implications of Thioredoxin Reductase Inhibition

TR inhibition can be expected not only to boost intracellular levels of hydrogen peroxide, but to make this hydrogen peroxide more effective for modulating cell signaling pathways, as thioredoxin functions to reverse the oxidation of protein sulfhydryl groups which hydrogen peroxide induces [20,21]. In particular, hydrogen peroxide tends to activate the stress-activated MAP kinases, JNK and p38, via ASK-1; thioredoxin functions to inhibit ASK-1 activation [22]. When p53 is activated by DNA double-strand breaks-as occurs during PARP inhibitor therapy in p53-competant cells – JNK activity interacts with

p53 to encourage apoptosis, as opposed to cell cycle inhibition or senescence [23]. This effect would thus tend to boost the cell-killing efficacy of PARP inhibition.

TR inhibition also suppresses NF-kappaB activity, as the transcriptional activity of this factor is dependent on its reduction by thioredoxin [24-26]. Constitutively elevated NF-kappaB promotes aggressiveness and chemoresistance in many cancers [27]. It also promotes sequestration of intracellular iron, by inducing ferritin heavy chain synthesis [28,29]. The iron stores of many cancers are elevated, but ferritin expression prevents this iron from catalyzing oxidative damage-including DNA single-strand breaks-both by sequestering labile iron, and by oxidizing it to ferric form [29-31]. Hence, inhibition of NF-kappaB activity by auranofin could be expected to increase the availability of intracellular labile iron, particularly in cancers that have high constitutive NF-kappaB activity.

Curiously, auranofin is now being studied clinically in ovarian cancer-a cancer for which PARP inhibition has particular promise [32-35]-because this drug also inhibits protein kinase C- α , a novel PKC which promotes malignant behavior in many ovarian cancers [36,37]. In a recent phase II study, in which the standard anti-inflammatory dose schedule for auranofin was employed, 4 out of 10 treated patients maintained a stable CA-125 for at least a month, with one patient achieving a reduction in this marker; hence, the authors of the study concluded that auranofin merited further evaluation in ovarian cancer [37].

These considerations suggest that joint therapy with the recently-approved PARP inhibitor olaparib, DCA and auranofin might have interesting potential as a cancer control strategy for cancers that are somewhat responsive to PARP inhibition. This combination could be tested in cell culture studies and in tumor-bearing rodents; if the addition of DCA and auranofin potentiated the killing efficacy of olaparib in these models, without notably enhancing systemic toxicity, clinical evaluation could be contemplated.

Tetrathiomolybdate Can Suppress Superoxide Dismutase Activity

Induction of single-strand breaks via oxidative stress could also be promoted by treatment with the copper chelator tetrathiomolybdate (TM), an orphan drug employed in the treatment of the genetic disorder Wilson's disease [38,39]. Copper plays a supportive role in angiogenesis [40,41] and rodent studies demonstrate that partial copper depletion -sufficient to maintain plasma ceruloplasmin at 15-20% of baseline levels-slows angiogenesis and cancer xenograft growth in mice [42-46]. This strategy has proved to be reasonably well tolerated in cancer patients, and Phase I and II studies in various types of cancer provide suggestive evidence that it may indeed retard tumor growth in some cases-though Phase III studies have not yet been done to confirm this [47-55].

One effect of copper depletion is to reduce the activity of the copper-zinc-dependent cytoplasmic form of superoxide dismutase (SOD1). Studies in tumor-bearing rodents indicate that tolerable levels of TM-induced copper depletion do indeed decrease SOD1 activity in tumor, endothelial cells, and blood cells; this effect apparently contributes to the tumor-retardant effect of TM therapy, as concurrent administration of an SOD-mimetic drug partially inhibits TM's efficacy in this regard [41,56,57]. It is reasonable to expect that suppression of cytoplasmic SOD activity in cancer cells would increase the rate of single-strand breaks via the Haber-Weiss reaction, whereby

superoxide, hydrogen peroxide, and labile iron collaborate in the generation of hydroxyl radical [58]. Notably, in cancer cells defective for homologous recombination owing to deletion of RAD54B, SOD1 inhibition induces DNA double-strand breaks and apoptotic death [59,60]. This phenomenon may be more general to cancer cells expressing "BRCA-ness", and presumably would be amplified by concurrent PARP inhibitor therapy. These considerations suggest that TM therapy could enhance the efficacy of concurrent olaparib therapy, and moreover slow cancer growth by an independent impact on angiogenesis.

Intravenous Ascorbate as an Adjuvant to PARP Inhibition

An additional pro-oxidative strategy with potential for boosting the efficacy of PARP inhibitor therapy is high-dose intravenous ascorbate, which has already been studied in ovarian cancer [61,62]. In mill molar concentrations that are achievable by high-dose infusion, ascorbate generates hydrogen peroxide in the extracellular space; cancers tend to be selectively susceptible to killing by this hydrogen peroxide, likely because their intracellular levels of superoxide and labile iron tend to be relatively high [62-66]. Ascorbate therapy could be expected to promote DNA single-strand breaks somewhat selectively in cancer cells, and hence would seem likely to boost the killing efficacy of concurrent PARP inhibition. However, intravenous ascorbate therapy can only be applied episodically, and PARP inhibition kills cancer cells primarily in S phase, when double-strand breaks develop during DNA synthesis. Hence, only a fraction of the tumor's cells would be targeted during a single ascorbate infusion session. That's why measures which achieve a continuous elevation of cancer oxidative stress - such as DCA and auranofin - might importantly complement the tumor kill achieved by episodic ascorbate infusions.

Summing Up

These considerations suggest that certain agents which are currently clinically available, and which can amplify cellular levels of reactive oxygen species - including DCA, auranofin, TM, and parenteral ascorbate - have the potential to boost the efficacy of PARP inhibition as a strategy for treating the many cancers which have an impaired capacity for homologous recombination. The rate at which PARP inhibitors induce apoptosis in such cancers will hinge on the rate at which single-strand breaks occur; oxidant stress can increase production of such breaks. Other oxidant-generating drugs currently in development -such as elesclomol - may be worthy of evaluation in this respect once they become available [67]. This concept should be readily testable in cell culture studies, and in mice bearing human xenografts of cancers defective for homologous recombination.

To the extent that the measures recommended here can be expected to induce oxidative stress somewhat selectively in cancers, they would not be expected to increase the killing of healthy tissues, and hence should be rather well tolerated. The fact that baseline oxidant production is often greater in cancers than in healthy tissues, may imply that these agents will achieve a greater level of oxidant stress in such cancers. For example, whereas TM therapy would be expected to reduce SOD activity in all tissues, the impact of this on oxidant stress should be greater in cells that are making increased amounts of superoxide. Analogously, auranofin will boost hydrogen peroxide levels more dramatically in cells whose mitochondria have defective

respiratory chains and hence make more superoxide – a common characteristic of cancer cells. And DCA could be expected to boost mitochondrial oxidant production most in cancer cells that have the Warburg phenotype and hence express elevated PDK activity.

In contrast, the use of PARP inhibitors to potentiate cytotoxic chemotherapy is likely to amplify the toxicity of the chemotherapy, as cytotoxic agents damage DNA in cancer cells and healthy cells alike; clinical experience in Phase I trials tends to bear this out [68,69]. Hence, there may be little net therapeutic gain in when PARP inhibitors are used during chemotherapy. (This however does not rule out the potential value of maintenance PARP inhibitor therapy as a follow-up to chemotherapy [70].)

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