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[DCA: A Promising treatment that can target cancer cells]

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[Radiation and chemotherapy are the most common and effective methods of treating cancer. There is a problem with these treatments however as they can have severe side effects because they can damage other types of cells and not just cancer ones. This paper looks at treatments that can target cancer cells specifically while leaving normal cells alone. There are many possible ways to do this because cancer cells have differences to normal cells. One way this is done is by targeting the different metabolism of cancer cells. For example, unlike normal cells, cancer cells get most of their energy from glycolysis so that would be a good target. One promising drug that could possibly do this is Dichloroacetate (DCA). One study in this article has shown that DCA can switch the metabolism of cancer cells from glycolysis to mitochondrial respiration which causes these cells to undergo apoptosis while leaving normal cells unharmed. Another promising part of DCA is that it has been used in humans with brain cancer and no toxicity has been observed. Finally, this review looks at a promising treatment using this drug as a part of a platinum compound. More trials and testing need to be done however and the amount of DCA you can give a patient might vary due to the genetics of the patient.]

Introduction

Targeted cancer therapies are therapies that aim to stop the spread and growth of cancer by targeting specific targets that only appear in cancer. These therapies could potentially lower the damage and side effects other therapies have on patients such as chemo or radiation therapy because they can't really differentiate between what cells they are damaging. One aspect of cancer that can be targeted from different cells is its metabolism. "The glycolytic phenotype in cancer appears to be the common denominator of diverse molecular abnormalities in cancer and may be associated with a (potentially reversible) suppression of mitochondrial function" (Michelakis et al 2008). Cancer has a different metabolism than normal cells so If we target this we can select these cells and leave normal cells alone. Research is being done on a very promising drug that can do this called Dichloroacetate (DCA). DCA supposedly works by switching the metabolism of cancer cells from glycolysis to mitochondria respiration (Michelakis et al 2008). This is important

because "early observations concerning the metabolic differences between cancer and normal cells showed that cancer cells are inherently dependent on glycolysis for production of chemical energy" (Madhok et al, 2010). The activation of this pathway which is normally not active in certain cancer cells seems to cause them to undergo apoptosis. The reason DCA can do this is because it is an inhibitor of pyruvate dehydrogenase kinase. "The inhibition of pyruvate dehydrogenase kinase increases the flux of pyruvate into the mitochondria, promoting glucose oxidation over glycolysis which also reverses the suppressed mitochondrial apoptosis in cancer". (Michelakis et al 2008). One study in this article aimed to "investigate the effects of DCA on the growth of colorectal cancer cells in an attempt to examine PDK inhibition as a novel therapeutic strategy against colorectal cancer" (Madhok et al, 2010).

So, what about using DCA with other drugs? One promising aspect of cancer treatment is the use of platinum compound drugs that target cancer. "Targeted delivery and controlled release of inactive platinum (Pt) prodrugs may offer a new approach to improve the efficacy and tolerability of the Pt family of drugs, which are used to treat 50% of all cancers today" (Dhar et al 2010). This study aimed to test the effectiveness of DCA when used as a part of a platinum compound. The compound they created is called Mitaplatin, which is a fusion of DCA and cisplatin. The study hypothesized that "DCA released inside the cells by reduction of the platinum would simultaneously alter mitochondrial metabolism and deliver a dose of cisplatin" (Dhar et al 2010). So, just what is Mitaplatin and how does it work? Mitaplatin is a platinum IV Pt(IV) compound. Its mechanism of action is that after crossing the plasma membrane its negative intracellular redox potential reduces the platinum to release cisplatin, a Pt(II) compound, and two equivalents of DCA (Dhar et al 2010). In other words, after crossing the plasma membrane, mitaplatin becomes reduced to release both cisplatin and DCA. "By a unique mechanism, mitaplatin thereby attacks both nuclear DNA with cisplatin and mitochondria with DCA selectively in cancer cells" (Dhar et al 2010). They believed this would create a pretty effective platinum compound treatment as well as potentially getting past the resistance some cancers have to platinum compounds with the help of DCA. They tested a wide range of experiments with this drug to test its effectiveness at killing cancer cells while leaving normal cells unharmed.

Recent Progress

So, can DCA really be used to target and induce apoptosis in cancer cells while leaving normal cells alone? One study aimed to determine if "switching metabolism from glycolysis towards mitochondrial respiration would reduce growth preferentially in colorectal cancer cells over normal cells, and to examine the underlying mechanisms" (Madhok et al, 2010). To test this, they looked at how different cell lines reacted to being treated with DCA. The cell lines they used in this experiment were, "HB2 (breast epithelial cells of non-cancer origin), 293 (epithelial cells from human embryo kidney), HT29 (well-differentiated primary colorectal adenocarcinoma), SW480 (poorly differentiated primary colorectal adenocarcinoma), and LoVo (metastatic left supraclavicular lymph node from colorectal adenocarcinoma)" (Madhok et al, 2010). So all together they used 3 cancer cell lines and 2 non cancer cell lines. The first experiment they performed was to see if DCA could reduce cancer cell proliferation and if the effect was similar in normoxia and hypoxia conditions. They hypothesized that the influence of DCA would be particularly potent with oxygen levels that are insufficient to support additional oxidative phosphorylation. All cell lines (HB2, 293, HT29, SW480, and LoVo) were treated with a range of doses of DCA for 24–48 h in normoxic and hypoxic conditions (Madhok et al, 2010). The results of this experiment were that DCA did not affect the growth of cultures of the non-cancerous cells. There was however,

significantly reduced growth of cultures of all three colorectal cancer cell lines. Another important finding from this experiment was that there was little difference in the results in both normoxia and hypoxia conditions so the rest of the experiments were performed in normal oxygen conditions. The next experiment they performed was to investigate whether the reduced growth of cultures on treatment with DCA was associated with induction of apoptosis. "Cells were treated with range of doses of DCA (0, 10, 20, and 50 mM) for 24 and 48 h, and the proportion of cells undergoing apoptosis was assessed" (Madhok et al, 2010). They found that the amount of cancer cells that were killed increased with the amount of DCA and again found that there was little to no cell death in the non-cancerous cells. In another experiment they wished to examine whether the reduction in growth of cultures on treatment with DCA was associated with induction of growth arrest. "Cells were treated with 50 mM DCA for 24 or 48 h, and cell-cycle profiles were analyzed using flow cytometry assessment of DNA content after PI staining" (Madhok et al, 2010). DCA treatment caused changes in the cell-cycle profiles of all the cancer cells but had no effect on the non-cancerous cells. The most important experiment in this study they performed was to establish whether the changes in growth and apoptosis caused by DCA correlated with reduced glycolysis. To do this they measured lactate levels in growth media. This is because "Lactic acid is the end product of glycolysis and If DCA were inducing mitochondrial oxidative phosphorylation, pyruvate would be decarboxylated to acetyl-CoA and not reduced to lactate, hence lactate levels in the growth media would decrease" (Madhok et al, 2010). This experiment found that treatment with DCA reduced extracellular lactate levels in growth media in all the cancer and non-cancerous cell lines. Finally, they wanted to see what mechanism DCA uses to cause cells to undergo apoptosis. "To verify if the induction of apoptosis in cancer cells on treatment with DCA was associated with promotion of mitochondrial oxidative phosphorylation, we measured the intrinsic mitochondrial membrane potential ($\Delta\Psi m$)" (Madhok et al 2010). They wanted to measure the membrane potential specifically because "escalation of mitochondrial respiration would reactivate the electron transport chain and reduce the hyperpolarized $\Delta\Psi m$ in cancer cells." (Madhok et al, 2010).

So one study shows that DCA is correlated with apoptosis in vitro, but what about in vivo? Another study actually tested this on patients diagnosed with recurrent malignant brain tumors (RMBTs). A phase 1 trial was conducted by an "open-label study of oral DCA in 15 adults with recurrent WHO grade III – IV gliomas or metastases from a primary cancer outside the central nervous system" (Dunbar et al 2014). The main objective of this study was for the "detection of a dose limiting toxicity for RMBTs at 4 weeks of treatment, defined as any grade 4 or 5 toxicity, or grade 3 toxicity directly attributable to DCA" (Dunbar et al 2014). Fifteen patients were enrolled in this study between 2009 and 2013 and were assigned a DCA dose based on their body weight. The genetics of the patients was also taken into consideration when assigning doses of DCA. 'Because individuals who harbor at least one wild type (EGT) haplotype for GSTZ1/MAAI metabolize DCA more rapidly than those who lack this haplotype, the protocol was amended to a priori stratify (i.e., not randomize) patients into one of two cohorts: 1) EGT carriers; and 2) non-EGT carriers (Dunbar et al 2014). Several patients had to drop out of this study due to unrelated reasons so not all of the patients completed the trial. The results of this experiment found that All subjects completing at least 1 four-week cycle remained clinically stable during this time and remained on DCA for an average of 75.5 days (Dunbar et al 2014). The study concluded that DCA's safety and tolerance are supported by protocol compliance of the eight evaluable patients and the average duration of survival of the five patients who died (Dunbar et al 2014).

One study found some interesting results from the potential use of DCA in a platinum compound. The first experiment they performed was to test the cytotoxicity of Mitaplatin on In Vitro Cellular Assays. In this experiment they used mitaplatin on several cell lines and compared the results to the cytotoxicity of another Pt(IV) compound as a control as well as with DCA and cisplatin by themselves. The results were that Mitaplatin was established to have cytotoxicity comparable to that of cisplatin and had cytotoxic levels that exceed almost all known Pt(IV) compounds (Dhar et al 2011). The next experiment was to test the ability of Mitaplatin to cause apoptosis in cancer cells. "To investigate the ability of mitaplatin to promote apoptosis in cancer cells by a mitochondrial-regulated mechanism, changes in the mitochondrial transmembrane potential ($\Delta\psi m$) of cancerous NTera-2 and healthy normal fibroblast cells before and after mitaplatin treatment were investigated by two assays" (Dhar et al 2011). With this analysis, detectable apoptosis was found and quantified in the cancerous cells while no detectable apoptosis was found in the normal cells. The next experiment was to see the anticancer activity of cisplatin to attack DNA by "the formation of intrastrand 1,2-d(GpG) cross-links" using fluorescently stained antibodies (Dhar et al 2011). Fluorescence was observed so these results confirm that mitaplatin has "dual cell-killing modes involving DCA, which destroys mitochondrial function, and cisplatin, which simultaneously impedes DNA-mediated processes in the nucleus" (Dhar et al 2011). In order to be a good targeted therapy mitaplatin needs to selective kill cancer cells and not effect healthy cells. "We therefore treated a coculture of normal fibroblasts and cancerous NTera-2 cells with mitaplatin, cisplatin, or a mixture of one equivalent of cisplatin and two equivalents of DCA" (Dhar et al 2011). The results of this experiment were that cisplatin and DCA killed both the fibroblasts and NTera-2 cancer cells. Mitaplatin on the other hand selectively killed only the cancer cells. The final experiment was to see if mitaplatin would work on cisplatin resistant cells. Interestingly the cisplatin resistant cells displayed a low level of resistance to mitaplatin compared to cisplatin. The other platinum compound that was used as a control was not as effective.

Discussion

So, just what does all of these studies mean for DCA as a potential targeting drug that can? First a discussion on their effectiveness must be looked at. The results of the first study showed that DCA did not reduce growth of non-cancerous cells but caused significant decrease in cancer cell proliferation. They also proved that the cause of this was because DCA inhibits PDK which attenuates glycolysis and turns on mitochondrial oxidative phosphorylation. They did have a problem with the dosage of DCA they used. "However, the dose of DCA required to inhibit growth of colorectal cancer cells in our study is unlikely to be achieved clinically without causing significant side effects. The dose of DCA required to achieve the equivalent plasma concentrations in vivo would be about five to ten times than that used in clinical trials against lactic acidosis" (Madhok et al 2010). So in other words the dosage might be too high to use in humans.

The other study over brain cancer looked for toxicity although they didn't test how effective it was in actually treating the patient's cancer. In conclusion more testing should be done to determine if DCA can really be an effective drug to use in humans. The study over RBMTs concluded that DCA appears to be safe, tolerable and feasible for chronic administration in adults with RMBTs. The results of this experiment were that although some patients dropped out of this study due to unrelated reasons, the ones that did complete did not suffer any toxicity problems. "It should be noted however that two patients reported reversible sensory and motor peripheral neuropathy (PN) which has been directly associated with dose and duration of DCA administration in adults" (Dunbar et al 2014). This could be managed by changing the dose of

DCA. “These data indicate that oral DCA, administered within the dose range well-established in metabolic disease, is safe, well-tolerated and feasible for use in adults with RMBTs” (Dunbar et al 2014).

The study over mitaplatin concluded that the enhanced potency of mitaplatin is consistent with the expected dual killing mechanism. The results obtained in this study provide compelling evidence that mitaplatin can selectively kill cancer cells, leaving normal cells untouched. It could also be used for the treatment of cisplatin resistant as it showed increased effectiveness at causing apoptosis in resistant cells compared to cisplatin alone. So not only is DCA relatively safe to use in humans, it shows great promise in becoming a very effective targeted therapy that could hopefully limit the side effect of other cancer treatments.

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