Diskussions-Abschnitt aus:

A combination of alpha lipoic acid and calcium hydroxycitrate is efficient against mouse cancer models: Preliminary results

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The main finding is that a combination of alpha lipoic acid and hydroxycitrate strongly reduces tumor growth. Time to failure of animals is extended compared to those treated with cisplatin or 5-FU. There are differences in terms of apparent efficacy inbetween the three cancer models: the time to failure is reached later in the MBT-2 model than in the B16-F10, which is also reached later than in the LL/2 model. This can be related to the tumor development kinetics of each model: the MBT-2 bladder cancer evolve quite slowly as compared to the two others. Moreover, the tumor volumes were not the same at the beginning of the drug administration; and, an early treatment will be more effective than a later one.
Thus, we can not conclude from these preliminary data on the efficacy of the combination of drugs in relation to the different tumor types.

Cancer cells have a clearly altered metabolism. They display an increased uptake of glucose and, among other alterations, an increase in lactic acid production, and decrease in **pyruvate kinase** and **pyruvate dehydrogenase** activities, and an increase in **ATP citrate lyase** activity.

In this study, we targeted the last two enzymes. Pyruvate dehydrogenase complex (**PDC**) is a large multi-enzyme complex composed of several copies of pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Pyruvate dehydrogenasekinase (PDHK) is an integral part of the PDC, with two or three PDHK dimers per complex. Inactivation of the E1 component of the PDC complex occurs when PDHK phosphorylates three serine residues on E1 (ser264, ser271 and ser203) (47).

It has been shown that **lipoic acid** restores the activity of **PDH** through the inhibition of PDHK (26). The fact that our results showed lipoic acid was able to inhibit tumor growth suggests that restoration of PDH activity allows **pyruvate** to be metabolized to **acetyl-CoA**, thereby reducing the amount of **lactate** produced by the tumor cells.

There is considerable evidence that production of lactate can provide an advantage to the growth of tumor cells, perhaps through creating an acidic environment which is damaging to normal cells (48,49). This is exactly the logic that was suggested by **McFate et al (50)**, who inactivated PDHK in a human squamous cell head and neck cancer cell line through the use of a specific shRNA thus apparently restoring the PDH activity, reverting the Warburg metabolic phenotype, and inhibiting growth of tumors obtained by xenografting these cells into nude mice.

Several studies suggest that lipoic acid can indeed inhibit the growth of cancer cells that show a glycolytic phenotype. A publication by **Van de Mark et al (51)** reported that lipoic acid initiated apoptosis in the FaDu and Jurkat human cell lines as well is in a Ki-v-Ras transformed Balb/c-3T3 murine mesenchymal cell line where apoptosis was not induced in normal cells. Similarly, when HT-29 human colon, H460 human lung epithelial cancer cells as well as two hepatoma cell lines (murine FaO, human HepG2 and SMMC-7721 cells) and human promyelocytic HL-60 cells were exposed to lipoic acid, apoptosis was observed (**52-56**).

ATP citrate lyase (ACL) is a key enzyme involved in **lipid bosynthesis**, but only recently has it been linked to the Warburg effect. Perhaps the first study to explore the role of ACL in the production of lipids in cancer cells was published by **Hatzivassiliou et al (22)**. These authors showed that inhibition of ACL, using both siRNA and the specific inhibitor SB-204990, was able to inhibit cancer cell growth both in vitro and in vivo, using xenografts in nude mice, and that in general levels of ACL are significantly elevated in these tumor cell lines.

A rather large assortment of different cell lines was investigated. Cancer cells displaying high rates of glucose metabolism are more severely affected by the two treatments, whereas those displaying a low rate of aerobic glycolysis are largely unaffected, indicating that **the ability of ACL inhibition to suppress tumor growth correlates with the glycolytic phenotype** of the tumor. It is important to note that ACL is the enzyme that converts **citrate** to **acetyl-CoA**, which is then used as the building block for lipid synthesis.

To the best of our knowledge, no experiment demonstrating **hydroxycitrate** efficacy against cancer has ever been published, although one in vitro study demonstrated that calcium hydroxycitrate capacity to interfer with the pyruvate metabolism in tumorigenic cells (**57**).

This study examined pyruvate metabolism in four cell lines, two tumorigenic cell lines (H.Ep2 and ESH TRI-1) a suppressed cell line (ESH p6), and MRC5, an untransformed diploid fibroblast cell line from a fetal lung. Treatment of these cells with hydroxycitrate resulted in a marked decrease (4-6-fold) of pyruvate consumption in the two tumorigenic cell lines but about a two-fold increase in the MRC5 cells. However, the percentage of pyruvate that was oxidized to CO 2 was increased nearly two-fold. In addition, a marked decrease in lactate production was also noted following hydroxycitrate treatment.

This is consistent with the recent results of **Wellen et al (58)** demonstrating that one gene that appeared to be down-regulated through the inhibition of histone acetylation that occurred following the inhibition of ACL was lactate dehydrogenase A.

Therefore, inhibition of ACL may not simply deprive tumor cells of lipids required for cell proliferation, it may also result in down-regulation of lactate dehydrogenase, decreasing aerobic glycolysis and allowing pyruvate to be imported into the mitochondria where it can be metablized through the Krebs cycle.

Therefore, it would appear that **the approach utilized in this study may indeed result in a synergistic attack on the metabolic pathways** used by these cancer cells. **Lipoic acid inhibits the activity of PDHK**, thereby allowing pyruvate that is imported into the mitochondria to be converted to mitochondrial acetyl-CoA by PDH, thus allowing the Krebs cycle to proceed, while **hydroxycitrate inhibits ACL**, which reduces the lipid available for cell proliferation and decreases the activity of lactate dehydrogenase.

Our results suggest that targeting cellular metabolism may be **an alternative to conventional cytotoxic chemotherapy**.

Neither hydroxycitrate nor lipoic acid are known to target DNA or to be cytotoxic to normal human cells (59,60). **No demonstrable toxicity** due to the long-term combination of these two drugs could be observed during this study.

Lipoic acid and calcium hydroxycitrate, when **administrated alone**, have at best a cytostatic effect on tumor cells (**Fig. 1)**. The rate of response is slow, clearly different from conventional chemotherapy. When the **drugs are combined**, however, there is a major cytotoxic effect similar to results obtained with conventional cytotoxic chemotherapy. In vitro, cancer cells are killed within hours when exposed to cytotoxic drugs (**61**).

In the three animal models studied, the kinetics of response to the combined metabolic treatment is similar to the positive controls (5-FU or cisplatin). Using either conventional

chemotherapy or metabolic therapy, **it takes at least one week** to obtain a decrease in the rate of tumor growth.

Metabolic therapy seems effective in delaying tumor growth in the models studied, although there are **differences in the individual responses**. It should be noted that the choice of dose for this first animal study was empirical. We did not demonstrate any toxicity at doses as close as possible to the plasma level reached when humans were treated either by food supplements or in case of lipoic acid when treated for neuropathy.

Both lipoic acid and calcium hydroxycitrate are well known to the clinician and **are available over the counter.**

Since the early 1990s, **lipoic acid** has been widely used as a dietary supplement, typically at doses in the range of 100-200 mg/day. It has been approved as a treatment for diabetic neuropathy in Germany. The dose is usually **600 mg per day**, but higher doses have been prescribed (**62)**.

Experimental and clinical studies have indicated the potential usefulness of lipoic acid treatment as a therapeutic agent for the prevention of various pathologies including diabetes, atherosclerosis, ischemia-perfusion injury, degenerative processes in neurons, joint diseases, radiation injury, heavy metal poisoning and HIV activation (**54**). Furthermore, lipoic acid has been used in clinical trials as an antioxidant to fight cancer-related cachexia and oxidative stress syndrome (**63,64**), and a case-report showed that in association with selenium and low-dose naltrexone, lipoic acid was efficacious in the long-term survival of a patient diagnosed with poorly differentiated adenocarcinoma of the pancreas (**65**). The therapeutic potential of lipoic acid as well as mechanisms of action has recently been reviewed (**66**).

Hydroxycitrate, extracted from **Garcinia cambogia** fruit, is used as a weight loss agent because it presumably limits lipogenesis and reduces food intake, although the results obtained on human body weight regulation are controversial (**67**). In humans, it is usually administered at **1500 mg/day**, but 3 g/day has already been tested (68).

One strength of this study is the **full randomization** with both passive and active controls. In addition statistical tests were **corrected for the fact that multiple comparisons** were made. However, we acknowledge that **the lack of evaluation of enzyme activity is a limitation** as it made it impossible to establish and measure the effect of the experimental treatment on the targeted enzymatic pathways.

Cancer is characterized by a large number of metabolic disturbances which are linked to cancer progression, as highlighted by two recent publications (**69,70**). The present study shows that targeting **just one** of these disturbances is not particularly effective, but targeting **two** has a striking effect.
It is tempting to assume that targeting a **third** metabolic alteration will lead to an even more effective response. In conclusion, **this study demonstrates the efficacy of targeting metabolic pathways of cancer cells to control their proliferation** and may provide incentive for further investigation to clarify the mechanisms at work. It is a first step towards the possibility of developing a metabolic approach to cancer therapy.