Potent anti-cancer agents, non-redox-active lipoate derivatives, have pervasive, catastrophic regulatory effects on cancer cell metabolism.

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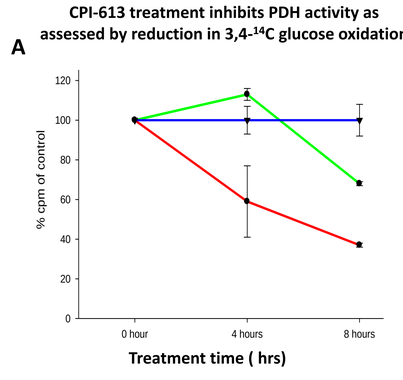
Lipoic acid is necessary for the catalytic function of four mitochondrial enzymes: Pyruvate dehydrogenase (PDH), α-Ketoglutarate dehydrogenase (KGDH), Branched chain keto acid dehydrogenase (BCKD) and the glycine cleavage system (GCS). Lipoate catalytic intermediates also represent regulatory signals apparently influencing mitochondrial carbon flux. We previously showed that non-redox-active lipoate analogs ("thioctoids") induce regulatory phosphorylation of the PDH E1α subunit, a known lipoate-sensitive regulatory process (Zachar, et al., 2011). Thioctoids powerfully inhibit tumor metabolism and efficiently induce complete cell death in every tested tumor cell line (op.cit.). Moreover, thioctoids have potent in vivo anti-cancer efficacy in human tumor xenograft models (op. cit).

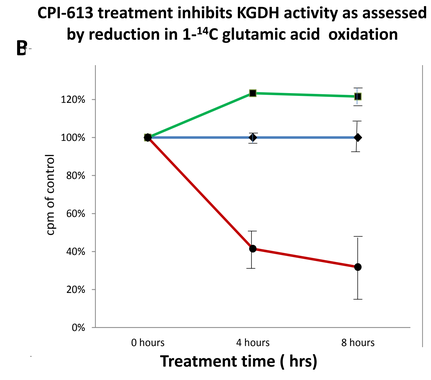
More recently we carried out an exhaustive metabolomic analysis of thioctoid effects and observe large perturbation of tumor cell mitochondrial metabolism. Here we present data on two of the lipoate containing enzymes (PDH & KGDH) and enzyme directly upstream of a third (BCKD), the acid branched chain amino transferase (BCAT). Further, perturbation of mitochondrial metabolism results in mitochondrial fragmentation and ultimately in cell death.

Collectively, our results indicate that thioctoids attack tumor mitochondrial metabolism on a broad basis. Apparently as a result of the central monitoring/regulatory role of lipoate, these analogs generate "false" regulatory signals, shutting down tumor mitochondrial metabolism and induce tumor cell death.

Thioctoids inhibit PDH and KGDH activities

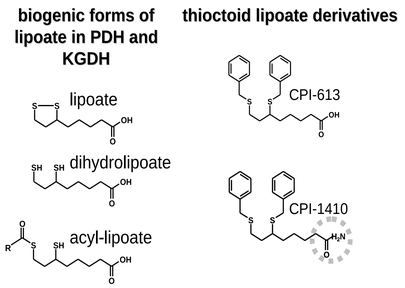


A. PDH activity in glucose medium was monitored by following oxidation of 3,4-^{14}C glucose. PDH activity is reduced in a time and dose dependent manner.

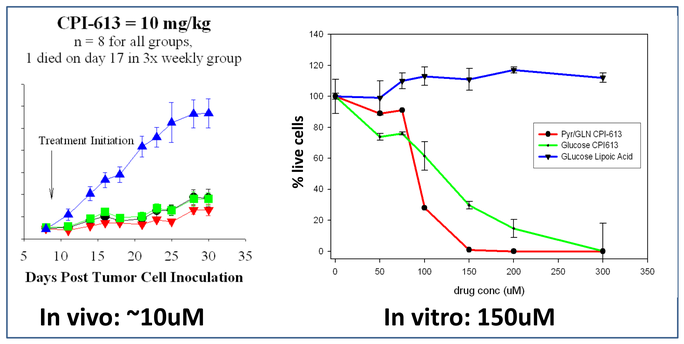


B. KGDH activity in glucose medium was monitored by following oxidation of 1-^{14}C glutamic. KGDH activity is reduced in a time and dose dependent manner.

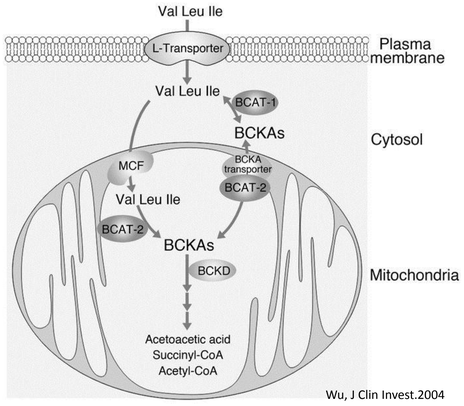
Lipoic acid and Thioctoids



Thioctoids inhibit tumor growth in xenografts and are cytotoxic in *in vitro* tissue culture models



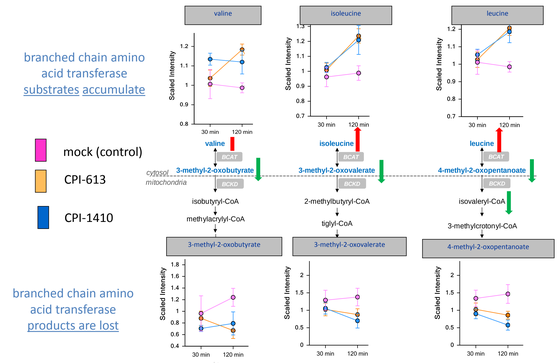
Thioctoids are at least 10X more potent in vivo than in vitro.



Wu, J Clin Invest.2004

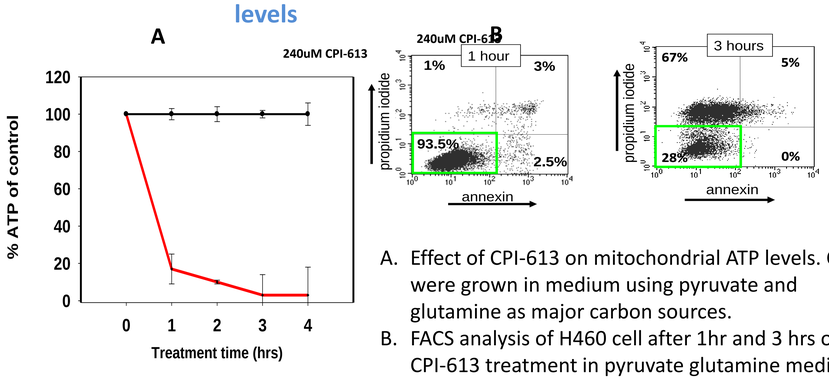
Schematic of Branch Chain amino acids (BCAA) catabolism

Thioctoids inhibit BCATs, upstream of BCKD



Metabolomic analysis of BCAA catabolism indicates that thioctoid treatment affects BCAA transferase(s), (BCATs)

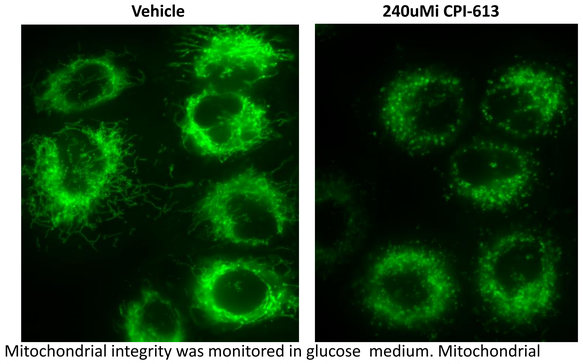
Thioctoids rapidly reduce mitochondrial ATP levels



A. Effect of CPI-613 on mitochondrial ATP levels. Cells were grown in medium using pyruvate and glutamine as major carbon sources.

B. FACS analysis of H460 cell after 1hr and 3 hrs of CPI-613 treatment in pyruvate glutamine medium.

Thioctoids induce mitochondrial fragmentation



Mitochondrial integrity was monitored in glucose medium. Mitochondrial fragmentation is both dose and time dependent. First signs of fragmentation appear after one hour of treatment and are complete by three hours (shown).

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