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# Inhibition of SCOT and Ketolysis Decreases Tumor Growth and Inflammation in the Lewis Cancer Model

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## Abstract

We tried with the Lewis cancer model on mice, an inhibitor of succinyl-CoA: 3- oxoacid-CoA transferase (SCOT), a specific ketolysis enzyme. This inhibitor acetohydroxamic acid (AHA) studied by Pickart and Jencks, was also given together with compounds inhibiting enzymatic steps upstream and downstream of SCOT. We show that an inhibition of ketolysis slows down the growth of tumors, as measured by evaluating their volumes, in comparison to negative and positive control groups. In addition, the increase of NFkB transcription and inflammation, a hallmark in cancer progression, markedly decreases after SCOT and ketolysis inhibition. Since these results may question the beneficial effects of prescribed ketogenic diets, we tried to propose a possible explanation, indicating that ketone bodies formed in fasting and those resulting from high fat diets, may not have the same impact on the fatty acid synthesis needed by proliferating tumor cells.

**Keywords:** SCOT Inhibition, Ketolysis in Tumor, Acetohydroxamate, Lewis Cancer Model, Nfkb Inhibition

## Introduction

In tumor cells, an abnormal phosphorylation of Pyruvate kinase (PK) M2 isoform [1,2] and of Pyruvate dehydrogenase (PDH) [3] inhibits both PK, the last enzyme of glycolysis, and PDH at the entry in oxidative glycolysis, starting the citric acid cycle. In order to overcome these two bottlenecks, and recover energy, large amounts of glucose ferment, triggering a "Pasteur Effect", as for yeast cells in anaerobic situations. However, since the glycolytic entry of Acetyl-CoA in oxidative metabolism is narrow as well, tumor cells will ferment glucose into lactic acid even in the presence of oxygen (Warburg Effect) [4,5]. On the other hand, we know that mitotic tumor cells have to make new membranes and need for this fatty acids and lipids. In this process, the citrate condensation reaction starting the Krebs-cycle, which is elevated in tumor cells [6] condenses Acetyl-CoA and Oxaloacetate to form citrate. Then the efflux of citrate from mitochondria feeds the cytosolic fatty acid synthesis pathway "via" ATP citrate lyase and Acetyl-CoA carboxylase (ACC). When the fatty acid synthesis is active, malonyl-CoA the product of ACC automatically turns-off their degradation, because malonyl-CoA inhibits the fatty acid canityl transporter of mitochondria. This blocks the mitochondrial degradation of fatty acids by beta-oxidation into Acetyl- CoA, if cytosolic fatty acid synthesis is ON [7]. Thus, with both glycolytic and fatty acid sources of Acetyl-CoA turned-off, tumor cells become dependent of ketone bodies for synthesizing their mitochondrial Acetyl-CoA, by the ketolysis of betahydroxybutyrate (BHB). This process depends of a specific enzyme: succinyl-CoA: 3- oxoacid-CoA transferase (SCOT) the product of the OXCT1 gene. The SCOT enzyme has two substrates: first succinyl-CoA, which comes from alphaketoglutarate, or from glutamine and glutamate, whiles the second SCOT substrate acetoacetate, comes from BHB provided by ketogenesis, taking place essentially in the liver. Branched chain amino acids also contribute to ketogenesis. The enzyme SCOT is specific for ketolysis, it will form Acetoacetyl-CoA then a thiolase gives Acetyl-CoA. This thiolase and BHB dehydrogenase that operate in ketolysis are the same as for ketogenesis, but work in the opposite direction. In the present work, we first tested on the Lewis cancer model, a forgotten nontoxic SCOT inhibitor, acetohydroxamic acid (AHA) for decreasing the ketolytic Acetyl-CoA supply to tumor cell mitochondria; we also inhibited enzymes upstream and downstream of SCOT, impairing the synthesis of lipid membranes of mitotic tumor cells. We show that decreasing the SCOT pathway decreases tumor volumes and the associated inflammatory process.

Our second goal was to understand some paradoxical observations related to the ketogenic diet. Indeed, several observations indicate that fasting, which elicits the release of glucagon and triggers ketogenesis, improves the situation of patients, which we would not expect, if Ketone bodies were a major Acetyl-CoA source for tumor cell mitochondria, feeding the lipogenesis of mitotic cells. Let us return to the initial observation of Brünings who invented the ketogenic diet, as recalled by Klement in 2019 [8]. We learnt that he gave it together with insulin injections, provoking a controlled hypoglycemia. We know that insulin elicits an influx of glucose in most cells and in pancreatic delta cells that will then release somatostatin, which inhibits glucagon and ketogenesis, depriving tumor cell mitochondria of Acetyl-CoA. In this very first part of Brünings' trial, he did observe a decrease of Tumors. Unfortunately, this effect did not last and a rebound of tumors took place after a while. Probably, the exhaustion of glucose and ATP in Delta cells hyperpolarizes them, through their $K_{ATP}$ channels, inhibiting the calcium dependent release of somatostatin. The decrease of somatostatin will then activate glucagon release, and boost ketogenesis, supporting the formation of Acetyl-CoA, which might explain the rebound of tumors observed by Brünings, particularly since patients received a high fat ketogenic Diet [9]. This still does not explain the difference between ketone bodies formed in fasting that seem favorable, and Ketone bodies associated to a high fat Diet. In relation to the present work, we propose a possible explanation for this ketogenic diet paradox.

## Materials and Methods

### Cells and Lewis Model

For evaluation of the anti-tumor effects of the below mentioned agents, we run a well-known xenograft Lewis model. Briefly, mice (6–7 weeks old inbred male C57BL/6J mice from Janvier-France) were divided in a weight normalized manner into the 10 groups housed five per cage with access to autoclaved mouse chow and water at libitum in a room under controlled temperature (22 °C), humidity (55%) and light (lights on 07h00 - 19h00). All animals received human care in compliance with the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. The LLC/1 cell-line CLS-400263 (Cell Line Service-Germany) cultivated in the medium DMEM Glutamax I (Gibco) with 10% of FBS (HyClone) and 1/10000 IU of Penicillin/Streptomycin (Sigma Aldrich P4333) were injected (implanted), into the subcutaneous

space of the left flank of the mice at 5 million cells per mouse in a 150µl shot and within 4 days of incubation. When tumors reached 110 mm3 of volume, mice were randomized, based to Tumor size (Millimeter) + Weight (Grams) and the treatments were started.

| **Agents and their concentrations** |
|---|
| Lipoic Acid (LIP) (Sigma Aldrich T1395) 10 mg/kg dissolved in PBS via Ethanol intraperitoneally (i.p.) two times per day (2X/D) |
| Calcium Hydroxycitrate (GAR) from Garcinia (IWDO-India) 250 mg/kg dissolved in PBS i.p. (2X/D) |
| Cisplatin (Sigma Aldrich 479306) 1 mg/kg dissolved in PBS i.p. (1X/2D) |
| AcetoHydroxamic Acid (AHA) (Sigma-Aldrich 159034) 225 mg/kg (LOW), 450 mg/kg (MEDIUM) and 900 mg/kg (HIGH) dissolved in PBS via Ethanol i.p. (1X/D) |
| Allicin (ALI) (Must be freshly synthesized from DADS) DADS: Diallyl Disulfide (Sigma-Aldrich SMB00378) 90 mg/kg dissolved in PBS-Ethanol i.p. (1X/D) |
| Epigallocatechin (EGC) (Sigma-Aldrich E3768 ) 150 mg/Kg dissolved in PBS i.p. (1X/D) |
| Lithothamnium calcareum (LIC) (Parchem - New Rochelle, New York) 180 mg/kg dissolved in PBS i.p. (1X/D) |

## Groups

| |
|---|
| Group 1: Zero Control injected just by culture medium and PBS |
| Group 2: Negative Control injected by PBS |
| Group 3: Positive Control injected by Cisplatin (Cis-Pt) |
| Group 4: Test 1 injected by AHA low concentration |
| Group 5: Test 2 injected by AHA Medium concentration |
| Group 6: Test 3 injected by AHA high concentration |
| Group 7: Test 4 injected by AHA/ALI Medium concentration |
| Group 8: Test 5 injected by AHA/ALI/EGC Medium concentration |
| Group 9: Test 6 injected by AHA/ALI/EGC/LIP/GAR Medium concentration |
| Group 10: Test 7 injected by LIC/ALI/EGC/LIP/GAR |

## Tumor Volume measurements

Weights were measured three times weekly. Animals' behavior was monitored daily. Resulting tumors were measured by calipering (Absolute Digital Digimatic Vernier Caliper) weekly for 7 weeks. Tumor Volume Formula was: Tumor Diameter 1 (Length) X Tumor Diameter 2 (width) X Tumor Height (Thickness) X 0.523. We stopped the treatments when the tumor dimensions (volume) in control group passed from tolerable volume in mice (3500 mm3).

## NFkB activity measurement

Inactive NFkB exists in a bound form to the inhibitory IkB proteins in the cytoplasm. Treatment of cells with various inducers, such as LPS results in the degradation of the IkB proteins and the release of NFkB dimers, which subsequently translocate to the nucleus where they activate appropriate target genes. We measured p65 nuclear translocation (NFkB activation), in the tumor homogenates re-suspended in 1 ml hypotonic lysis buffer (20 mM HEPES, pH 7.5, 5 mM NaF, 10 mM Na2MoO4, 0.1 mM EDTA and 250 mM p-nitrophenyl phosphate). After this step, 0.1 ml 10% Nonidet P-40 was added and after 10 minutes, the suspension was centrifuged, and the nuclear pellet was re-suspended in 0.2 ml complete lysis buffer (Active Motif, Rixensart,

Belgium). After re-suspending the nuclear pellet in lysis buffer for 30 min with shaking, the lysates were centrifuged for 10 minutes at 14,000g at 4°C and the supernatant (nuclear cell extract) stored at –80°C. The protein concentration was determined by a MicroBCATM protein assay (PIERCE, Rockford, IL). Five µg of nuclear extracts were tested for the NFkB activation by using the NFkB p65 TransAM transcription factor assay kit (Active Motif, Carlsbad, California USA) according to the manufacturer's instruction (www.activemotif.com). Briefly, the nuclear extracts were subjected to binding of NFkB p65 to an immobilized consensus oligonucleotide (5'-GGGACTTTCC-3') in a 96-well plate. Active form of NFkB p65 specifically binds to this sequence and primary and HRP-conjugated secondary antibodies were added. A standard protein panel of seven increasing concentrations of recombinant NFkB P65 (Active Motif, Carlsbad, California USA) was prepared using the following concentrations: 0.008, 0.0156, 0.0312, 0.0625, 0.125 and 0.5 ng/µl. After the colorimetric reaction, the samples were measured in a spectrophotometer at 450 nm.

## Results

### Targeting the ketolytic pathway at SCOT, plus upstream and downstream enzymatic steps

The SCOT inhibitor found by Pickart and Jencks: Acetohydroxamic (AHA) [10] (lithostat) is in use for treating bladder stones and urine infection; it inhibits the urease of bacteria, by complexing the metal of the enzyme as other siderophores. The list of siderophores from bacterial or other origin is vast, and we should indeed try to find the better ones. Biological organisms use siderophores for finding in the environment the ferric iron they need for their development, in competition with other organisms. There are also the interesting mitoketocines developed by Lissanti's team [11]. Another interesting SCOT inhibitor is Pimozide [12] this compound used in Psychiatry, displays anti-cancer properties. We also find in the list of histone deacetylase inhibitors (HDAC)s potential SCOT inhibitors that derive from hydroxamic acid with clear anti-tumor effects. In the present work, we tested on the Lewis mouse cancer model, AHA the SCOT inhibitor studied by Pickart and Jencks [10]. The Figure 1 situates the SCOT enzyme in relation to the citric acid cycle. It explains that the SCOT dependency of tumor cells, results from the interruptions of the two other sources of mitochondrial Acetyl-CoA, the glycolytic source at the PK and PDH bottlenecks, and the fatty acid source turned-off by malonyl-CoA formed along the lipogenic pathway. In the experiments we present, we tried to act on SCOT with AHA, and upstream of SCOT with epigallocatechin (EGC), which inhibits the monocarboxylate transporter (MCT) of Betahydroxybutyrate (BHB) and its uptake by the cell [13]. Moreover, EGC inhibits glutamate dehydrogenase (GDH) [14], which should limit the formation of succinyl-CoA and substrate supply to SCOT. In earlier works, we observed that one could affect tumor growth with octreotide a somatostatin equivalent that decreases glucagon, acting upstream on ketogenesis. Downstream of SCOT, the enzyme thiolase could also be a possible target. Further, ahead we again used two previously tested compounds, Lipoic acid (LIP) and Hydroxycitrate from Garcinia cambodgia (GAR). We designated them Metabloc (Mtbl) as in previous work [15-16]. They decrease cytosolic lipogenesis by limiting the citrate efflux from mitochondria, and inhibit ATP citrate lyase in the cytosol. We also gave allicine (ALI) for inhibiting cytosolic Acetyl-CoA synthetase fed by external acetate [17,9]. We should recall that epigallocatechin (EGC) inhibits Fatty acid synthetase (FAS) acting also downstream of SCOT. In the present experiments, we tested three doses of AHA (high, med, and low): groups 6, 5, and 4 of five mice each bearing implanted tumors. Then in another group, we added Allicin (ALI) to the medium (med) AHA dose, (AHA med, plus ALI): group 7. Another group received (AHA med, plus ALI, plus EGC): group 8. The next group received (AHA med, plus ALI, plus EGC, plus Mtbl): group 9. Then, we tried to replace AHA by another compound with siderophore and anti-cancer properties: Lithotamnion calcaneum from red algae (LIC) [18], in this group LIC, instead of AHA, was associated to the other compounds (LIC, plus ALI plus EGC, plus Mtbl) Group 10. We compare all the groups to the cisplatin (Cis- Pt) positive control for the Lewis model, which efficiently decreases the tumor volume (2.68 fold) at the end of the experiment. Table 1 gives the raw data mean tumor volumes for the different groups and standard deviation, with t test statistics and P value significance. Figure 2 presents the results normalized in percentage of the Cis-Pt positive control. As far as tumor volumes are concerned, inhibiting the SCOT pathway reaches a 65% to 75 % decrease.

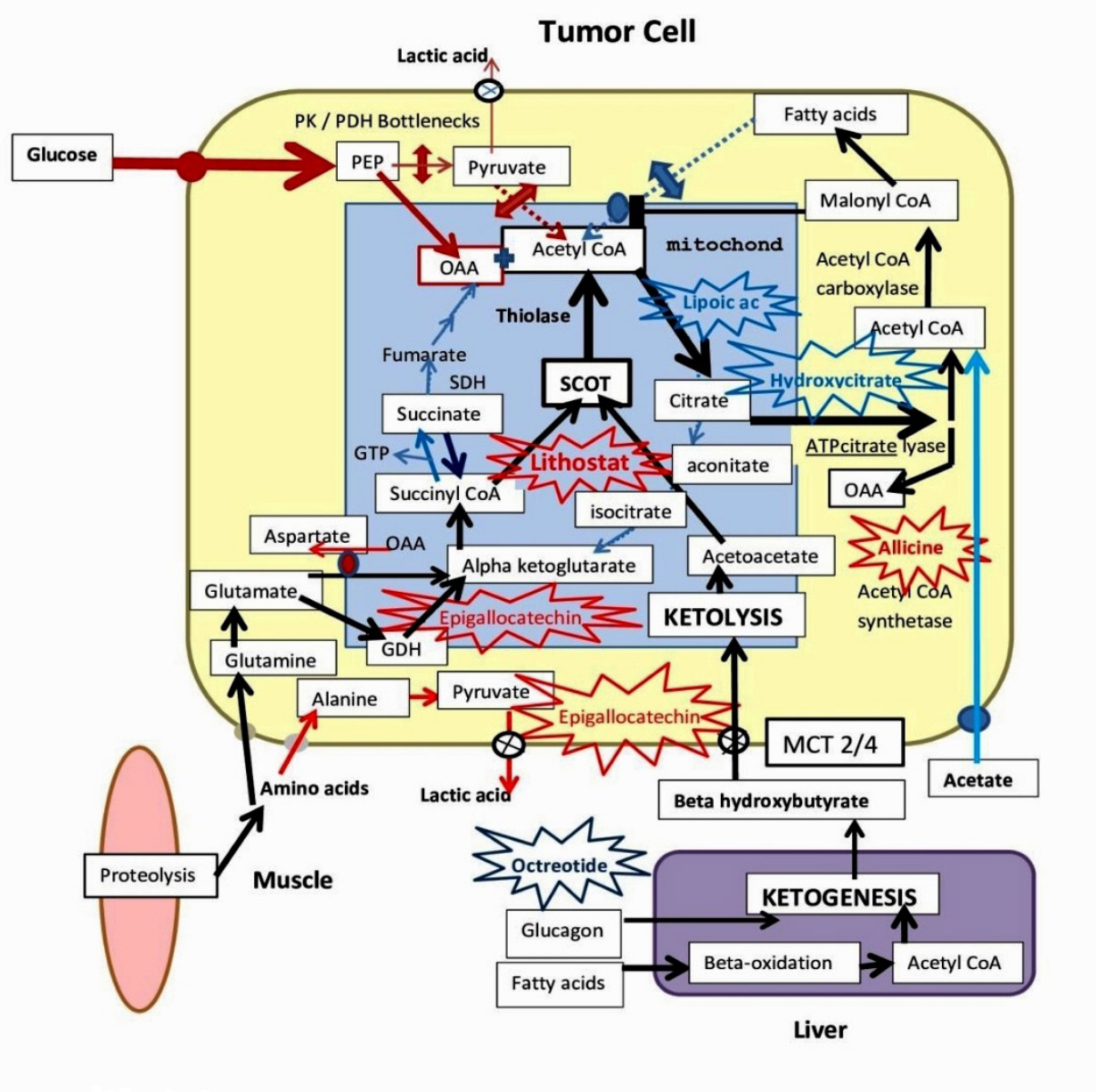


**Figure 1:** Inhibitiors and targets for SCOT dependent tumors

Acetohydroxamic acid (AHA) lithostat, is a typical SCOT inhibitor. Upstream of SCOT, Epigallocatechin( EGC) from green tea, decreases Glutamate dehydrogenase (GDH), and inhibits the TMC transporter of betahydroxybutyrate (BHB). Downstream of SCOT, Alpha R lipoate (LIP) decreases citrate condensation (via NADH reduction), while Garcinia cambodgia-hydroxycitrate, (GAR) inhibits ATP citrate lyase; the mixture LIP plus GAR was designated metabloc (mtbl) in previous works. Moreover, EGC is known for decreasing fatty acid synthetase (FAS). Finally, allicine (ALI) inhibits the direct external acetate supply to acetyl-CoA synthetase in the cytosol. We selected these essentials: 1- Lithostat; 2-Epigallocatechin; 3- alpha R lipoic acid; 4- Garcinia Cambodgia (hydroxycitrate); 5- Allicin. In red, the new targets, in blue those previously tested, including octreotide tested in another work [16]. Note that the ketolytic-SCOT pathway provides mitochondrial Acetyl-CoA, since there is an interruption of its glycolytic and fatty acid sources in tumor cells.

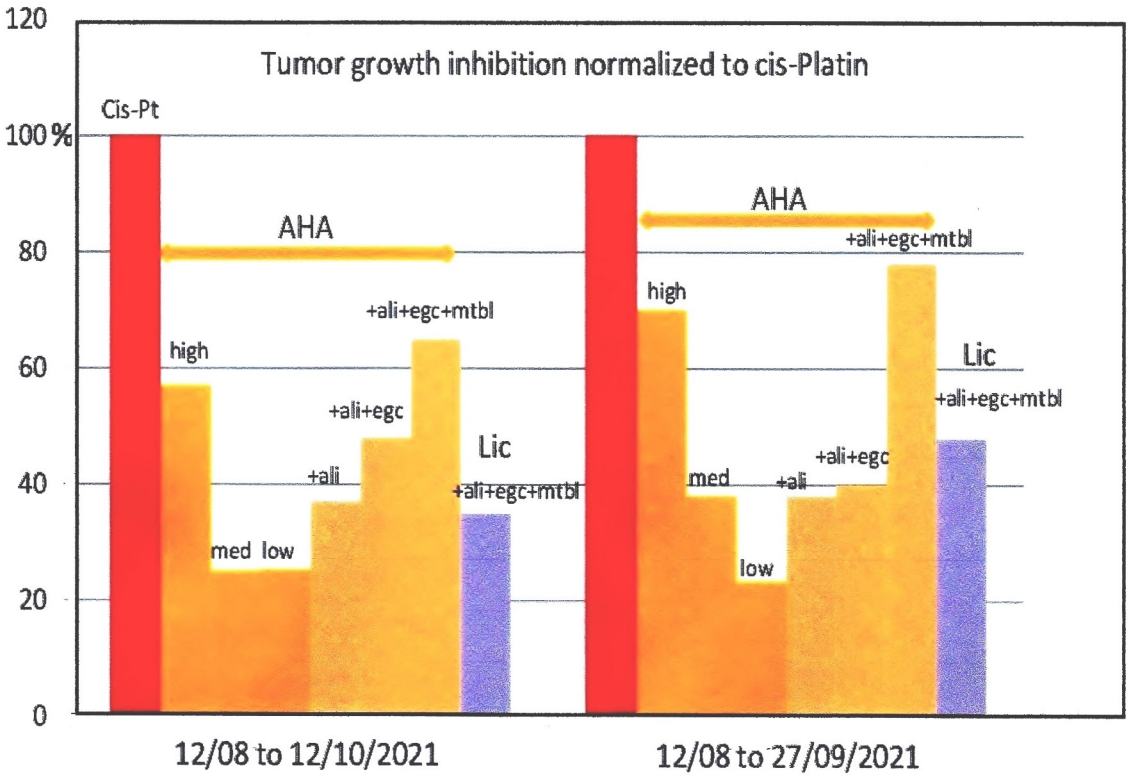


**Figure 2:** Tumor volume decrease, in percent of the positive cisplatin control

The additive effect of the different compounds tested for inhibiting SCOT and the ketolytic pathway are represented in percent of the cisplatin (Cis-Pt) positive control using the Lewis mouse cancer model. The left diagram gives the values at the end of the experiment. The right diagram gives the values at an earlier stage (dates indicated). On the left diagram, we did not discriminate between low and medium dose of acetohydroxamic acid (AHA) but the high dose was above reaching 55% of the positive control. Using the medium dose (med), we tested the additions of Allicine (Ali) then of Epigallocatechin (Egc), then metabloc (mtbl), which designates lipoic acid (lip) plus Garcinia hydroxy citrate (Gar). The effect reaching reaches 65% of the positive control. In the last column AHA was replaced by Lithothamnion from red algae (LIC) reaching 35 % of the Cis-Pt control. The right diagram differentiated well the AHA doses, the complete mixtures for AHA and LIC, reaching 75% and 50% respectively of the positive Cis-Pt control.

**Table 1:** Effect of Ketolysis inhibition on the decrease of tumor volumes

| **12/08/2021** Injected tumor cells | **Tumor volume. mm3** | **Cis Pt** | **AHA** | | | | | | **LIC** |
|---|---|---|---|---|---|---|---|---|---|
| | | | Low | med | high | med+ Ali | med + Ali +Egc | med+ Ali +Egc + mtbl | +Ali+Egc+mtbl |
| **27/09/2021** | 1627 +- 130 | 871+- 81 | 1450+- 91 | 1347+- 89 | 1101+- 58.8 | 1334+- 61 | 1320+- 87 | 1036+- 125 | 1256+- 29.7 |
| **t-statistic N=5** | | P<0.0001 | P=0.0373 | P=0.0041 | P<0.0001 | P=0.0018 | P=0.0023 | P=0.0001 | P=0.0003 |
| **12/10/2021** | 3893+- 454 | 1451+- 144 | 3370+- 156 | 3282+- 399 | 2505+- 209 | 2989+- 202 | 2728+- 98 | 2304+- 297 | 3041+- 97 |
| **t-statistics N=5** | | P<0.0001 | P=0.048 | P=0.0537 | P=0.0003 | P=0.0036 | P=0.0005 | P=0.0002 | P=0.0034 |
| **Tumor growth Inhibition normalized to Cis-Pt effect** | 27/09 | 100% | 23% | 38% | 70% | 38% | 40% | 78% | 48% |
| | 12/10 | 100% | 22% | 25% | 57% | 37% | 47% | 65% | 35% |

At the end of the trial, all tumors were homogenized and tested for NFkB nuclear transcription. We know that aberrant and constitutive NFkB activation has been detected in many human malignancies [19], the nuclear translocation of NFkB in the tumor cells, supporting proliferation and inflammation. By inhibiting the SCOT pathway, we observe a decrease of NFkB nuclear translocation, measured as indicated in methods, on samples of five µg protein of the nuclear lysate, for the different groups. The effect of SCOT inhibition, on the decrease of NFkB nuclear translocation was better than for the Cis-Pt positive control. The table 2 gives the raw data, mean NFkB translocation and standard deviation, for the different groups plus t test statistics and significance. The figure 3 represents the inhibition of NFkB transcription normalized to the Cis- Pt positive control. The inhibition of nuclear NFkB translocation resulting from SCOT inhibition was twice better in the AHA full group, and 1.5 better in LIC full group, than for the Cis-Pt positive control (Figure 3).

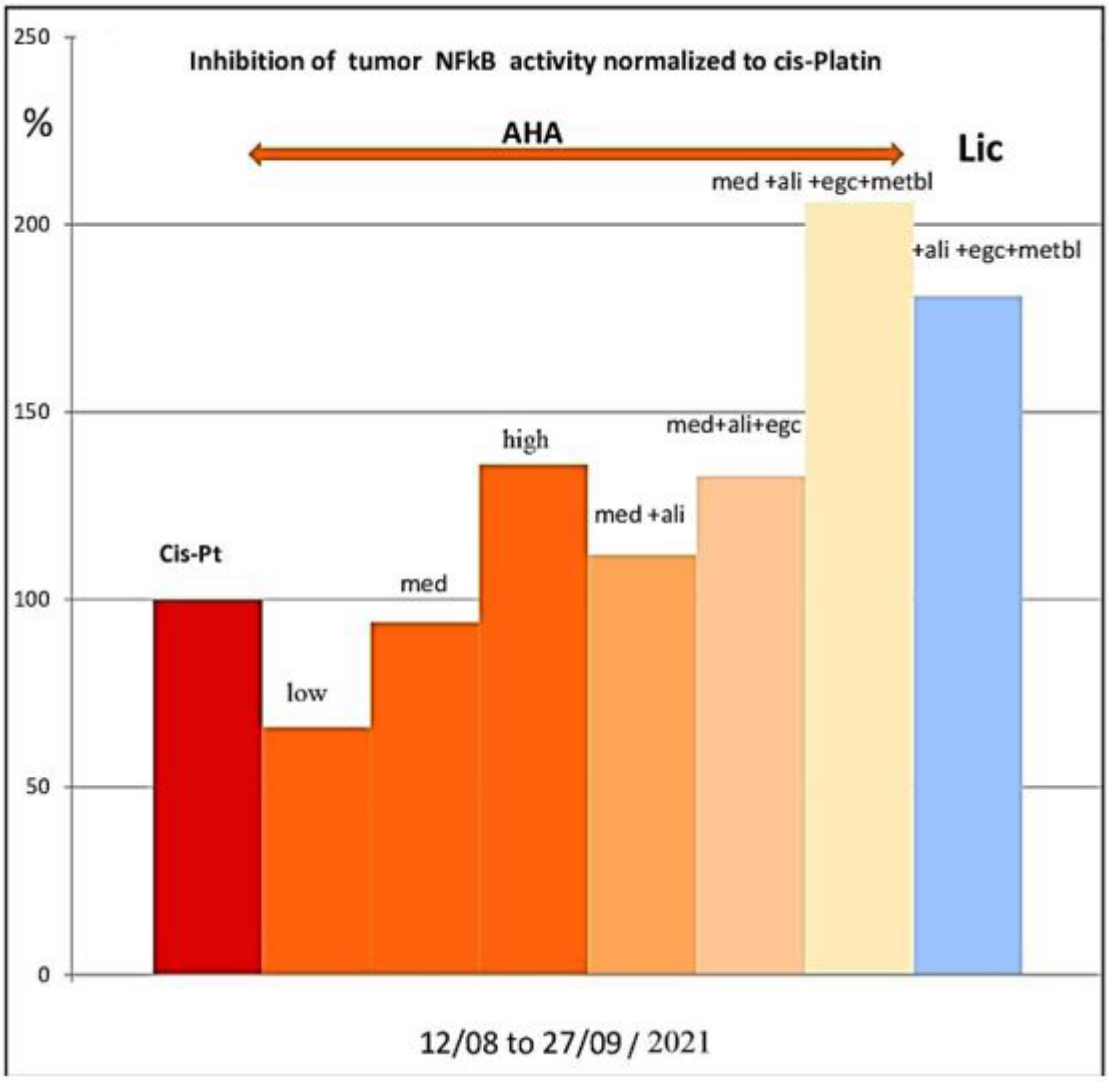


**Figure 3:** NFkB transcriptional inhibition, by SCOT and ketolysis inhibition

AHA the SCOT inhibitor markedly decreases NFkB nuclear translocation, for the low, med, and high AHA doses. We successively observe the additive effect of allicine (ali) epigallocatechin (egc) and metabloc (mtbl), designating lipoic acid, plus Garcia hydoxycitrate; added to the medium AHA dose (med), as indicated for the different columns of the diagram. In the last column, we replace AHA by lithothamnion (LIC) keeping the other compounds. The inhibition of NFkB nuclear translocation is shown in percent of the Cisplatin (Cis Pt) decrease of the positive control. Here, the effect of SCOT and ketolytic pathway inhibitors, on NFkB transcriptional inhibition, was greater than for the positive cisplatin control, reaching 200% for AHA and 150% for LIC, with all additives, as indicated above each column.

**Table 2:** Effect of Ketolysis inhibition on the decrease of NFkB nuclear activity in tumors

| **12/08/2021** Injected tumor cells | **Tumor NFkB ng/µl nuclear activity** | **Cis Pt** | **AHA** | | | | | | **LIC** |
|---|---|---|---|---|---|---|---|---|---|
| | | | Low | med | high | med+ Ali | med + Ali +Egc | med+ Ali +Egc + mtbl | +Ali+Eg-c+mtbl |
| **12/10/2021** | 0.452+-0.0312 | 0.305 +-0.0322 | 0.352+-0.0225 | 0.315+-0.0225 | 0.251+-0.0201 | 0.286+-0.0112 | 0.256+-0.0278 | 0.146+-0.0256 | 0.181+-0.0479 |
| **t-statistic N=5** | | P=0.0001 | P=0.0004 | P<0.0001 | P<0.0001 | P<0.0001 | P<0.0001 | P<0.0001 | P<0.0003 |
| **Tumor NFkB Inhibition Normalized to Cis-Pt decrease** (33%) | 12/10 | 100% | 66% | 94% | 136% | 112% | 133% | 206% | 181% |

## Discussion

### Effect of SCOT and ketolysis inhibition, on the decrease of tumor volumes and inflammation

The experiments indicate that inhibiting SCOT and ketolysis reduces tumor volumes by a factor reaching 60% to70% of the Cis-Pt positive control. However, on another tumor associated parameter, NFkB nuclear translocation, the effect of SCOT and ketolysis inhibition, was above the Cis-Pt effect, reaching a 200% inhibition for NFkB nuclear translocation. It is probable that normal immune cells, and macrophages of the host, invade the implanted tumor, these cells are not dependent as the tumor cells upon ketolysis and SCOT for making their Acetyl-CoA, since they have other Acetyl-CoA supplies than ketolysis. However, they do contribute to the tumor volume. By inhibiting SCOT and ketolysis one affects preferentially tumor cells, leading to a decrease of tumor volumes, below the Cis-Pt positive control, which affects all cells present in the tumor, including immune cells that do not depend upon ketolysis. On the other hand, SCOT inhibition decreases NFkB translocation, for all cells present in the tumor, immune cells and macrophages. Presumably, decreasing ketolysis preserves the signaling action of BHB, which touches all cells in the tumor, eliciting an inhibition of NFkB transcription and inflammation.

In sum, Cis–Pt affects all cells, SCOT inhibition affects preferentially ketolytic dependent tumor cells, but suppresses for all cells, tumor cells and immune cells, the NFkB transcriptional message for proliferation and inflammation. We shall discuss below the dual action of ketone bodies, as nutrients for ketolytic dependent cells, and as signaling molecules controlling inflammation and proliferation.

### Nutrition and signaling by Ketones

Two other players are part of the game: First, the monocarboxylate transporters MCT 1/4 [20] that carry lactic acid out, and transport BHB in the cells. Second, a plasma membrane receptor, the hydroxyl carboxylic acid receptor (HCA2) [21] that transmits the signaling action of the ketone body BHB (Niacin is an agonist for this receptor). When BHB gets in the cell, it has several effects. First, BHB dehydrogenase will convert BHB into acetoacetate, feeding the SCOT pathway, needed by the tumor cell for making mitochondrial Acetyl-CoA. Second BHB is a HDAC inhibitor, favoring the expression of genes found in juvenile or embryonic cells (The M2 PK isoform for example). Third acetoacetate will stimulate IKK kinase and the phosphorylation of IKB, which liberates the transcription factor NFkB, eliciting its nuclear translocation and the expression of genes supporting inflammation and proliferation. The NFkB activity has indeed been involved in cancer progression [19]. By Inhibiting SCOT, the influx of BHB no longer pulled in through its MCT transporter will decline, impairing the growth of tumor cells. In parallel, the stimulation of IKK and NFkB nuclear translocation decreases. On the other hand, slowing down the MCT transporter, keeps more BHB out of the cell, its signaling effect on the HCA2 membrane receptor is preserved or increases. The activation of the HCA2 receptor by BHB is transmitted by Gi coupled receptor proteins, eliciting an inhibition of IKK kinase, which retains NFkB in the cytosol bound to IKB, decreasing inflammation and proliferation. This is true for both the tumor cells and the normal immune cells present in the tumor. The effect of HCA2 activation seems to depend of prostaglandins that inhibit the inflammasome in immune cell, decreasing the proteolytic activation of interleukins. In sum, if BHB gets in the cell through its transporter, it will favor tumor growth, *via* SCOT by providing Acetyl-CoA to tumor mitochon-

dria, and boost lipogenesis. We show here that blocking the ketolytic-SCOT pathway and its connection to lipogenesis, slows down the development of tumors. Moreover, by blocking the influx of BHB through its MCT transporter, one keeps more of it outside the cell, which will stimulate the HCA2 receptor signaling and suppress NFkB transcription, both for tumor cells, and for immune cells infiltrating the tumor, decreasing inflammation and proliferation. The inhibition of the SCOT pathway affected specifically tumor cells that depend of ketones for making Acetyl-CoA, which is not the case for normal host immune cells infiltrating the tumor, since they have other Acetyl-CoA sources. Whereas, for inflammation and proliferation, both immune cells, and tumor cells, responded to HCA2 signaling, activated by BHB kept outside the cell, by inhibiting its MCT transporter and SCOT, eliciting for NFkB transcriptional inhibition, an effect more elevated than for the Cis-Pt positive control.

## A possible explanation for the Ketogenic Diet paradox.

An interesting report shows that fatty acids of 16 carbons and above [22] coming with high fat diets, stimulate AMP deaminase, and boost the lipid synthesis pathway, because the decrease of AMP, suppresses the stimulation of AMP kinase, and its inhibitory action over ACC, which facilitates lipogenesis, and would then help the proliferation of tumor cells. Moreover, for incorporating fatty acids coming with a high fat diet, their transporter CD36 has to get in the plasma membrane, this will negatively regulate AMP kinase [23] and cancel its inhibitory action over ACC, which further activates ACC and lipogenesis. In contrast, Ketone bodies, formed in fasting do not stimulate AMP deaminase, particularly if complements such as eicosapentaénoique (EPA) and docosahexaénoïque (DHA) are given, decreasing thiolase and probably AMP deaminase, this holds back the citrate supply to lipogenesis, and preserves AMP kinase activity, which inhibits ACC and lipogenesis. In sum, the fasting ketone bodies are not equivalent to those produced by a high fat diet rich in saturated fatty acids above 16 carbons (Figure 4). This figure also illustrates the dual action of BHB entering in the cell through the MCT transporter, supporting tumor growth, and the signaling effect of BHB over the HCA2 receptor. By decreasing the influx of BHB through the transporter, SCOT inhibition impairs tumor growth, and NFKB mediated inflammation, this effect is amplified through an increased action of BHB on the HCA2 receptor, which further decreases NFkB transcription and inflammation (Figure 4).

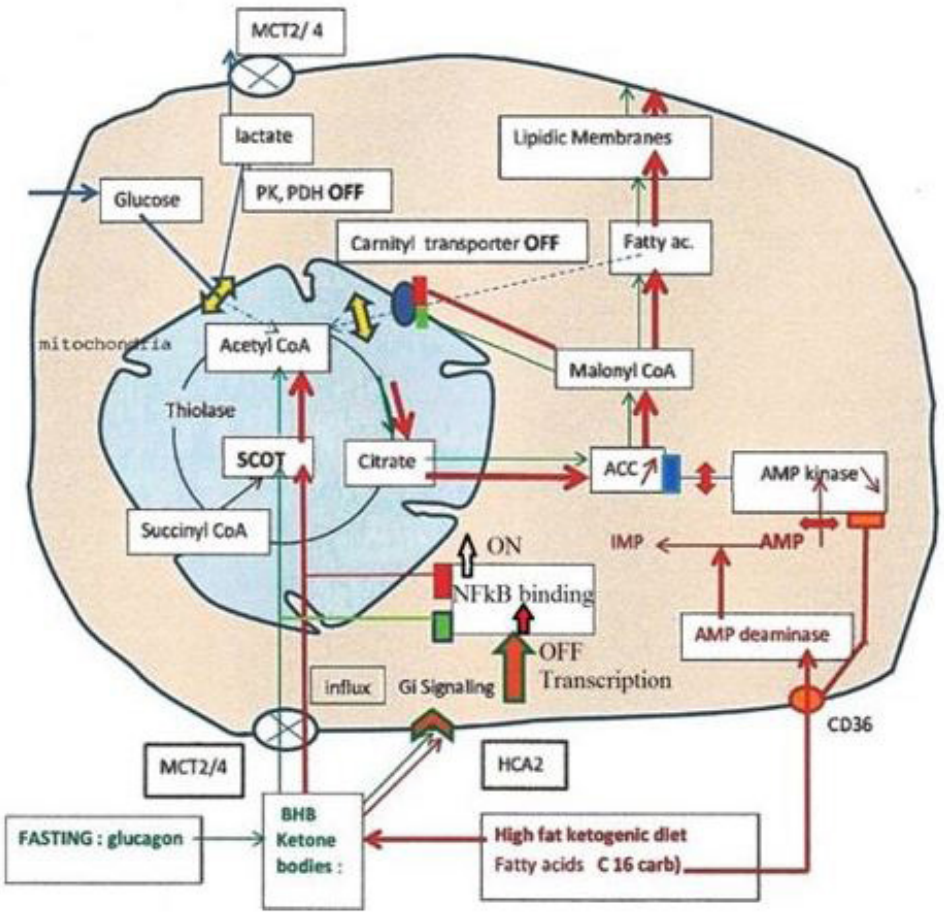


**Figure 4:** Tumor cells use more efficiently BHB, coming from a high fat diet, than fasting BHB

**Red pathway:** The influx of BHB through the MCT transporter increases, following a high fat ketogenic diet, since Fatty acids (C16 carbons and above) that come with the diet, stimulate AMP deaminase and boost lipogenesis, because the decrease of AMP, cancels the AMP kinase inhibition over ACC. In addition, the CD36 transporter of fatty acids inhibits AMP kinase, which facilitates even more lipogenesis. This will pull in more BHB through the MCT2/4 transporters and feed tumor growth. Green Pathway: In contrast, (BHB) formed in fasting is not associated with the intake of C16 fatty acids that stimulate AMP deaminase, which has a lower impact on fatty acid synthesis and lipogenesis, pulling in less BHB through the MCT transporters. Note the PK and PDH bottlenecks and the inhibition of the fatty acid carnityl transporter by malonyl-CoA, rendering tumor cells dependent of the supply of Ketone bodies, for making mitochondrial Acetyl-CoA. In Parallel, if BHB is taken-up through the MCT transporter, it will inhibit NFkB binding, eliciting NFkB transcription. On the contrary, the signaling action of external BHB on the HCA2 receptor favors the binding of NFkB (to its IKB partner) and turns OFF NFkB transcription. Inhibiting SCOT decreases the influx of BHB through the MCT transporter, impairing tumor growth by depriving it of mitochondrial Acetyl-CoA; in addition, NFkB triggered inflammation declines. However, this preserves the signaling action of BHB over the HCA2 receptor, which blocks even more NFkB transcription and inflammation.

We have recently suggested that ketolysis, which depends of SCOT-Oxct1, could be the "Achilles heel" of tumor cells [24]. We then gave examples of carcinogenic mutations of Krebs-cycle enzymes, in which one reinforces the supply of succinyl-CoA to SCOT [25]. This was the case of "Carney triad tumors", associated to a Succinodehydrogenase deficiency, which keeps more succinyl-CoA for SCOT [26]. We may even add here that tumors, optically detected by Protoporphyrin III and aminolevulinic acid accumulations, will feedback more succinyl-CoA to SCOT. Indeed, a mutation in the heme synthesis pathway downstream of Aminolevulinic acid synthetase, which condenses succinyl-CoA and glycine, would necessarily leave more succinyl-CoA for SCOT, supporting tumor metabolism. Moreover, recent works seem to have unraveled the regulation of OXCT1 expression in cancer. Apparently, long noncoding RNAs (lnc RNA) control the expression of genes. The finding that an antisense RNA silencer, (OXCT1-AS1) increases in several tumors, and silences the maturation of other small micro RNA regulators, such as Mir 886, is particularly interesting [27]. As other Micro RNAs, Mir 886 would bind to the 3' untranslated end of target mRNAs, and silence at the posttranscriptional level, the expression of proteins. Presumably, if the OXCT1-AS1 silencer increases and inhibits the expression and maturation of Mir-886, one would preserve SCOT and probably other proteins, supporting the dependency or tumors cells upon ketolysis, and promote their proliferation.

## Conclusion

The additive effects of some simple compounds, taken for other indications, or simply for comfort that inhibit SCOT and the ketolytic pathway, led to a decrease of tumor volumes, reaching 70 % of the cisplatin effect. Whereas, the decrease of NFkB nuclear translocation, elicited by SCOT and ketolysis inhibition by these compounds, was twice better than for cisplatin control, in this cancer model.

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## Conflicts of Interests

The authors declare no conflicts of interests.

## Author contributions

Following discussions with G. Tenenbaum and E. Berg on fasting and cancer, experiments for testing the dependency of tumor cells upon ketolysis became necessary. M. Israël proposed them for testing the published hypothesis. After approval by R. Abolhassani, he conducted the experiments in his laboratory (Nosco Pharm).

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