

Tumor regression with a combination of drugs interfering with the tumor metabolism: efficacy of hydroxycitrate, lipoic acid and capsaicin

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Summary Cellular metabolic alterations are now well described as implicated in cancer and some strategies are currently developed to target these different pathways. In previous papers, we demonstrated that a combination of molecules (namely alpha-lipoic acid and hydroxycitrate, i.e. Metabloc™) targeting the cancer metabolism markedly decreased tumor cell growth in mice. In this work, we

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demonstrate that the addition of capsaicin further delays tumor growth in mice in a dose dependant manner. This is true for the three animal model tested: lung (LLC) cancer, bladder cancer (MBT-2) and melanoma B16F10. There was no apparent side effect of this ternary combination. The addition of a fourth drug (octreotide) is even more effective resulting in tumor regression in mice bearing LLC cancer. These four compounds are all known to target the cellular metabolism not its DNA. The efficacy, the apparent lack of toxicity, the long clinical track records of these medications in human medicine, all points toward the need for a clinical trial. The dramatic efficacy of treatment suggests that cancer may simply be a disease of dysregulated cellular metabolism.

Keywords Cancer · Metabolism · Metabloc · Capsaicin · Warburg

Introduction

As has been pointed out in our previous publications [1–3], our laboratory has screened large numbers of compounds most of which have been clearly targeted at the altered metabolic pathways frequently present in cancer cells known as the Warburg effect. The alteration of glucose metabolism to produce lactic acid even under normoxic conditions, also known as aerobic glycolysis, was first described by Warburg almost 90 years ago [4]. Glucose metabolism in normal cells takes place through two distinct pathways; namely, glycolysis or the Embden-Meyerhof pathway, which produces pyruvate, followed by oxidative phosphorylation, the conversion of pyruvate to acetyl-CoA

to carbon dioxide and water in the mitochondria with the concomitant production of 34 molecules of ATP. The most likely hypothesis that explains this observation is because the cells utilize several glucose metabolic products as starting materials for the synthesis of key biomolecules, particularly lipids and nucleotides required for cellular proliferation [3, 5–7]. This provides cancer cells with a competitive advantage compared to normal cells.

Given the exposed arguments it is logical to target the altered metabolic pathway in order to inhibit cancer growth. As a consequence, it is not surprising that a rather large number of potential inhibitors of aerobic glycolysis have been evaluated both in vitro and in vivo as potential anti-cancer drugs [for reviews see 8, 9]. Despite some promising results, however, very few such inhibitors have been used clinically. Michelakis et al. [10] recently reported that the use of dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase (PDK), to treat glioblastoma multiforme in five patients led to tumor regression in three of these individuals. Berkson et al. [11, 12] have treated four pancreatic cancer patients with a combination of α -lipoic acid (alpha-LA) and naltrexone. Results were strikingly positive, and the first patient treated was alive and well 78 months following the initiation of treatment. alpha-LA is also an inhibitor of PDK, while naltrexone is an opioid receptor antagonist. Recently, CPI-613, an α -lipoic acid analog, was reported to be effective in some patients with pancreas cancer or myeloid leukemia [13, 14].

The limited clinical success of compounds that target the Warburg effect suggested that a single compound may not be effective, given the complexity of the aerobic glycolytic pathway. As a consequence, we adopted the strategy of identifying combinations of such compounds that are more effective than a single compound. The approach of treating cancer with combinations of standard chemotherapeutic drugs has become increasingly common, given the well-recognized plasticity of malignant tissue. A second key aspect of our strategy was to use compounds already in clinical use and that have been shown to be relatively safe.

Our first study [1] utilized a library of 27 compounds known to effect glucose metabolism drawn from a detailed literature analysis. In vitro tests were conducted on four cell lines at concentrations consistent with human dosage levels in order to assess antiproliferative activity. From the effective compounds, further in vitro testing was conducted on binary combinations and the seven combinations that showed significant activity in the in vitro tests were then evaluated in vivo against mice bearing a syngeneic MBT-2 bladder tumor. The most effective treatment was a combination of hydroxycitrate (HCA) and alpha-LA, which we have designated as METABLOCTM. The efficacy of this combination was confirmed using mice with B16-F10 melanoma and LL/2 Lewis lung. The HCA/alpha-LA

combination slowed tumor growth and increased survival with an efficacy similar to conventional chemotherapy [15].

Despite the promising results obtained with META-BLOCTM, tumor growth was only retarded. In order to improve this result a second library of 35 compounds was established using the same criteria to establish the first library to determine if addition of a third substance could improve the results. One of the substances evaluated, octreotide (OCT), although only moderately active on its own, provided an enhancement in both tumor growth and survival when combined with HCA and alpha-LA [2]. OCT is a well-known somatostatin analogue. It has been frequently used to treat neuroendocrine tumors in a long-acting release (LAR) formulation. Although its use has been generally restricted to relief of patient symptoms, there is evidence that OCT does indeed have anti-cancer effects [16].

Several of the drugs contained in the library of 35 compounds from which OCT was selected exhibited positive activity in vitro, but insufficient information regarding toxicity of these compounds had been obtained at that time to warrant in vivo evaluation. One of these compounds was capsaicin. A detailed search of toxicity data on capsaicin indicated that although there was some negative information regarding its toxicity, that there was sufficient information to assume that side effects of its use would be minimal. As a consequence, it was decided to evaluate both ternary combinations using CAP; namely, alpha-LA/HCA/CAP, but also the combination of CAP with all three compounds for which positive activity had been observed; that is, alpha-LA/HCA/OCT/CAP. As will be seen, results obtained with the ternary mixture were positive, showing increased tumor inhibitory effectiveness as compared to alpha-LA/HCA. The combination of all four compounds, however, was extremely effective.

Material and methods

Drugs

α -Lipoic acid, cisplatin and capsaicin were purchased from Sigma (St Quentin Fallavier, France) and hydroxycitrate (*Garcinia cambogia* extract containing 60 % hydroxycitrate as a mixture of 11 % calcium and 14.5 % potassium salts) was purchased from Indo World Trading Corporation (New Delhi, India). Octreotide (500 μ g/ml in lactic acid, sodium bicarbonate pH=4.2, water, mannitol) was obtained from Novartis (SandostatTM).

For in vivo experiments, the following doses were used: lipoic acid, 10 mg/kg, twice a day; hydroxycitrate, 250 mg/kg, twice a day [1, 2, 15]; capsaicin, 0.075 to 5 mg/kg, once a day; octreotide, 0.1 mg/kg, twice a day; and cisplatin,

1 mg/kg every other day. All drugs were diluted in saline solution (9 g/l) for intraperitoneal administration. Vehicle used for control groups was 0.5 % ethanol (lipoic acid and capsaicin solvent) in saline solution.

Animals

C3H (6 weeks old) and C57BL/6 mice (8 weeks old) were obtained from the “Centre d’Elevage Janvier”. The animals were maintained in accordance with the European community’s guidelines concerning the care and use of laboratory animals.

Tumor models

In all tumor models, 10^6 cells were inoculated in the back of mice: the lung carcinoma cells, LL/2 (ATCC number CRL-1642, purchased from LGC Promochem) in C57BL/6 mice; the bladder cancer cells, MBT-2, in C3H mice (MBT-2 are gift of INSERM group 03-33, Créteil, France); the melanoma cells, B16F10, in C57BL/6 mice. (See Table 1 for detailed descriptions of the models.) After randomization, treatments were intraperitoneally administered twice a day, either with a single drug or with a combination of several substances [1, 2, 15].

Outcome

For each study and each group, we present a mean of tumor volume with standard deviation (Fig. 1, 2, 3, 4 and 5. For analyzing in vivo tumor growth and response to treatment, we used two statistics explained by Supplementary Fig. 1: the ratio of change over time in median tumor volume in percent for groups treated with capsaicin and for control groups, T/C% (Suppl. Fig. 1 Left); the ratio dividing successes by failures of treatment with capsaicin across all possible pairs of mice, one coming from a treated group and the other from a control group, R_p (Suppl. Fig. 1 Right). As shown in Suppl. Fig. 1, a ratio $R_p = S_t/F_t$ lower than 1.0 would mean that the increase in the volume of the tumor is more often larger in the treated group than in the control group (less successes than failures); a

Table 1 Description of the studies for each cellular model

| Study (figure #) | Cellular model | Tumor volume at treatment start | Period of treatment | Mice per group |
|------------------|----------------|---------------------------------|---------------------|----------------|
| Fig. 1 | LLC | 101 mm ³ | day 12–32 | 9 |
| Fig. 2 | LLC | 102 mm ³ | day 14–35 | 9 |
| Fig. 3 | B16F10 | 117 mm ³ | day 10–31 | 9 |
| Fig. 4 | MBT2 | 93 mm ³ | day 11–32 | 9 |
| Fig. 5 | B16F10 | 100 mm ³ | day 17–71 | 4 |

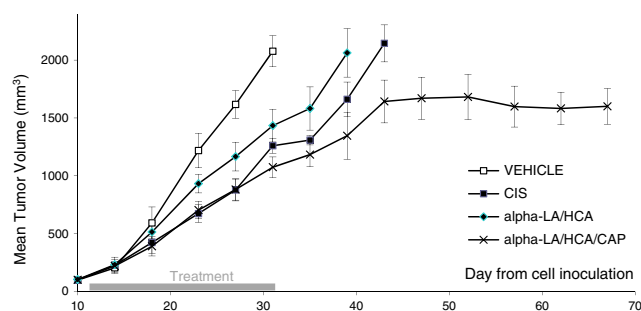


Fig. 1 Anti-tumor activity of different combinations of α -lipoic acid, hydroxycitrate and capsaicin on the Lewis lung carcinoma model. Mean tumor volume curves for each condition of treatment are compared to the references, vehicle and cisplatin. Legends: *alpha-LA* α -lipoic acid; *HCA* hydroxycitrate; *CAP* capsaicin; *CIS* cisplatin; grey bar treatment administration (days 12–32). Statistical details: see suppl. table 1

ratio larger than 1.0 would mean that the increase in the volume of the tumor is likely to be more often smaller in the treated group than in the control group (more successes than failures).

Animal weight and tumor size were measured twice a week. Tumor volume in mm³ was calculated from the measurement of two perpendicular diameters using a caliper according to the formula $l \times L \times h \times \pi/6$, where L and l are the largest and smallest diameters and h the height in mm, respectively [2]. Comparison of change in tumor volume due to treatment (T/C%) was determined as follows: 100 times median tumour volume increase of treated group/median tumour volume increase of control group (see Suppl. Fig. 1). Volume increase is obtained by subtracting the reference volume measured on the first day of treatment. Date of mouse death and date of tumor size measurement was systematically registered every 4th or 5th day. Animals were euthanized if cachectic or suffering. Observation ended by occurrence of death, when the tumor exceeded 2000 mm³ or after 70 days.

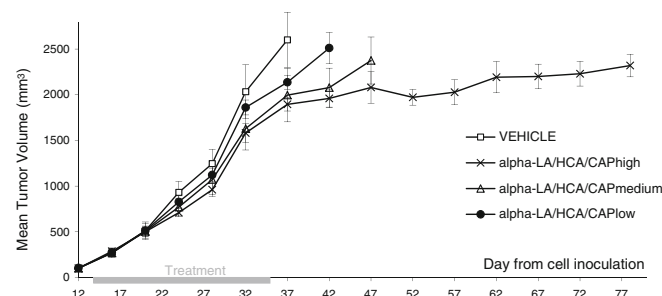


Fig. 2 Dose-dependent anti-tumor activity of the combination of α -lipoic acid, hydroxycitrate and capsaicin on the Lewis lung carcinoma model. Mean tumor volume curves for each condition of treatment are compared to the references, vehicle and cisplatin. Legends: grey bar treatment administration (days 14–35). Statistical details: see suppl. table 2

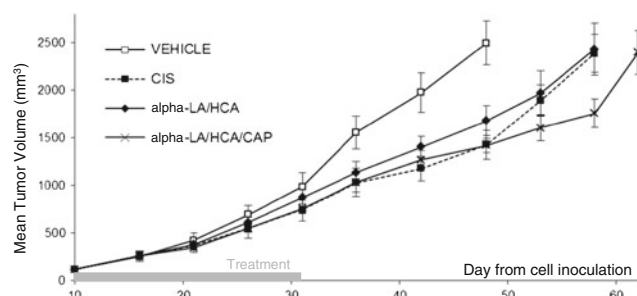


Fig. 3 Anti-tumor activity of the α -lipoic acid, hydroxycitrate and capsaicin combination on the melanoma model. Mean tumor volume curves for each condition of treatment are compared to the references, vehicle and cisplatin. Legends: grey bar treatment administration (days 10–31). Statistical details: see suppl. table 3

Data analysis

Description of tumor growth was produced for each day of measurement after the beginning of treatment. Each treated group got injections of a solution with capsaicin and each control group got injections without capsaicin. Graphical representation (Fig. 1, 2, 3, 4 and 5) was done by Excel. T/C% reported in Table 2 was computed at the middle of the follow-up of the control group from the beginning of treatment and at the end of the follow-up of control group. For each comparison between n_t mice treated with capsaicin and n_c control mice, the ratio of successes (S_t) divided by failures (F_t) was computed after comparing sorted Excel files (supplementary table 1 to 5). Under the null hypothesis, the probability distribution for all possible successes and failures follows a binomial with parameters $n = n_t \times n_c$ and $p=0.5$. For each comparison between one treated group and one control group, exact two-sided p-values were obtained from binomial distributions with appropriate n and $p=0.5$. Statistical significance of the difference between obtained T/C% and 100 % corresponds to the exact two sided p-value at the same date of measurement.

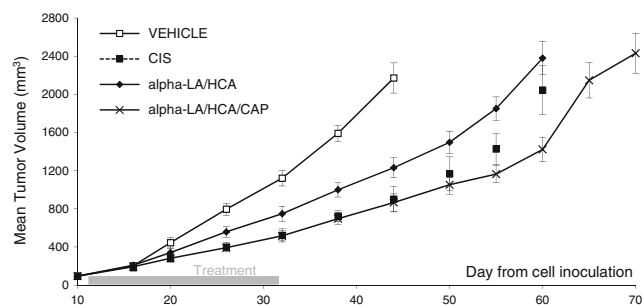


Fig. 4 Anti-tumor activity of the α -lipoic acid, hydroxycitrate and capsaicin combination on the bladder carcinoma model. Mean tumor volume curves for each condition of treatment are compared to the references, vehicle and cisplatin. Legends: grey bar treatment administration (days 11–32). Statistical details: see suppl. table 4

Results

The efficacy of the alpha-LA/HCA/CAP combination was first studied in the Lewis lung carcinoma model. Tumor volume development was analyzed (Fig. 1, Table 2, suppl. table 1). The alpha-LA/HCA/CAP combination was significantly more effective in inhibiting tumor development than was vehicle (T/C%=51 %; $p<0.000001$ at half-time). The difference with CIS was not significant at half time (T/C%=96 %; $p=0.51$ on day 27th), but it was significant after day 30th. The difference with alpha-LA/HCA was also significant (T/C%=74 %; $p<0.000001$ at half-time). In this study, the dose used for CAP (5 mg/kg/day) was quite high.

A second set of experiments in the LLC model assessed the efficacy of various doses of CAP (0.075, 0.75 or 5 mg/kg/day) while maintaining the dose of alpha-LA/HCA constant. The mouse group treated with the highest dose of CAP was significantly more effective in inhibiting tumor development than was vehicle (T/C%=70 %; $p<0.000001$ at half-time) (Fig. 2, Table 2 and suppl. table 2). The medium dose was less effective (T/C%=79 %; $p<0.000001$ at half-time) but better than the lowest dose which was (T/C%=83 %; $p<0.000006$ at half-time); however the differences were not major. We noticed that the efficacy of the highest dose of CAP was not as strong as was observed in the first experiment. Nevertheless, a dose-dependent anti-tumoral effect is observed.

We then studied the effectiveness of the alpha-LA/HCA/CAP combination using the B16F10 melanoma model (Fig. 3, Table 2 and suppl. table 3). As in the previous model, alpha-LA/HCA/CAP displayed a significant tumor development inhibition as compared to vehicle (T/C%=74 %; $p<0.000001$ at half-time). But, at half-time, this treatment was comparable to CIS (T/C%=99 %; $p<0.82$) and almost equivalent to alpha-LA/HCA (T/C%=89 %; $p<0.000017$). However, at the end of the follow-up (58 days), alpha-LA/HCA/CAP displayed a significant tumor development inhibition as compared to CIS (T/C%=74 %; $p<0.000001$) and alpha-LA/HCA (T/C%=66 %; $p<0.000001$).

In the fourth experiment, we studied the effectiveness of the treatment in the MBT-2 bladder carcinoma model (Fig. 4, Table 2 and suppl. table 4). The administration of alpha-LA/HCA/CAP significantly inhibited tumor development compared to vehicle (T/C%=41 %; $p<0.000001$ at half-time) or alpha-LA/HCA (T/C%=63 %; $p<0.000001$ at half-time). But, at half-time, this treatment was almost equivalent to CIS (T/C%=96 %; $p<0.00075$), while, at the end of the follow-up (60 days), it displayed a significant tumor development inhibition as compared to CIS (T/C%=67 %; $p<0.000001$).

In the last experiment, we assessed whether the combination of CAP with alpha-LA/HCA/OCT, the combination that had

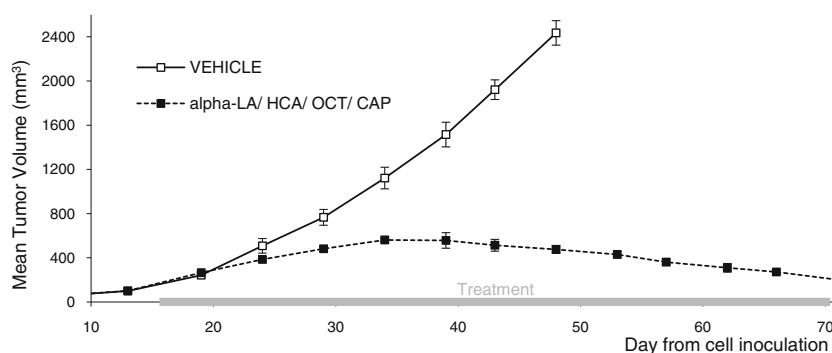


Fig. 5 Anti-tumor activity of the α -lipoic acid, hydroxycitrate, capsaicin and octreotide combination in the melanoma model. Mean tumor volume curves for each condition of treatment are compared to the references, vehicle and cisplatin. Legends: *OCT* octreotide; grey bar treatment administration (days 17–71). Statistical details: see suppl. table 5

provided the best results to date, would give even better results compared to the alpha-LA/HCA/CAP combination (Fig. 5 and suppl. table 5). Surprisingly, this quaternary combination was extremely effective: we obtained very significant tumor inhibition of tumor development compared vehicle at half-time ($T/C\%=44\%$; $p<0.00049$) and even better after 48 days of experiment ($T/C\%=32\%$; $p<0.00049$). The group treated survived up to 71 days after inoculation, when the study ended.

To summarize, our data demonstrates that the combination of METABLOC with capsaicin (alpha-LA/HCA/CAP) is of interest for the treatment of lung or bladder carcinoma and melanoma. The combination of four compounds (alpha-LA/HCA/CAP/OCT) is even more effective.

Discussion

For the past several years our laboratory has been evaluating combinations of clinically tested drugs that target glycolysis.

Our first study demonstrated the effectiveness of the combination of alpha-LA and HCA (designated as METABLOC™) in inhibiting the growth of three tumor models [1, 15]. In our most recent publication [2], we showed that a combination of α -lipoic acid (alpha-LA), calcium hydroxycitrate (HCA), and octreotide (OCT) reduced tumor growth in three different cancer models produced by syngeneic transplantation by almost 70 %. Despite this impressive result, this combination of compounds still allowed the three tumor models to continue to expand; that is, there was no evidence of tumor shrinkage. As a consequence, we attempted to identify a fourth compound that would lead to an actual reduction in tumor volume. Our current results indicate that the addition of capsaicin (CAP) to the three compounds previously evaluated did indeed lead to significant tumor shrinkage,

In our past studies, the alpha-LA/HCA/OCT ternary mixture was far more efficacious in inhibiting cellular proliferation in transplanted tumors than were any of the compounds individually or any binary combination. The

Table 2 Ratio of median tumor volume increase (T/C%) at half-time and at the end of each study

| Figure | Tumor Model | Treatment including CAP | T/C % by control group and delay (in days) | | | | | |
|--------|----------------|----------------------------|--|-----------|------------|-----------|--------------|-----------|
| | | | Vehicle | | Cisplatin | | alpha-LA+HCA | |
| | | | half-time | end | half-time | end | half-time | end |
| Fig. 1 | Lung cancer | CAP + alpha-LA + HCA | (23) 51 % | (31) 51 % | (27) 97 %* | (43) 70 % | (27) 74 % | (39) 65 % |
| Fig. 2 | Lung cancer | CAP low + alpha-LA + HCA | (24) 83 % | (37) 80 % | – | – | – | – |
| Fig. 2 | Lung cancer | CAP med. + alpha-LA + HCA | (24) 79 % | (37) 70 % | – | – | – | – |
| Fig. 2 | Lung cancer | CAP high + alpha-LA + HCA | (24) 70 % | (37) 70 % | – | – | – | – |
| Fig. 3 | Melanoma | CAP + alpha-LA + HCA | (26) 74 % | (48) 53 % | (36) 99 %* | (58) 74 % | (36) 89 % | (58) 66 % |
| Fig. 4 | Bladder cancer | CAP + alpha-LA + HCA | (26) 41 % | (44) 37 % | (38) 96 % | (60) 67 % | (38) 63 % | (60) 60 % |
| Fig. 5 | Melanoma | CAP + alpha-LA + HCA + OCT | (34) 44 % | (48) 32 % | – | – | – | – |

NB: Half-time day depends on actual measurement from the beginning of treatment

alpha-LA α -lipoic acid; HCA hydroxycitrate; CAP capsaicin; OCT octreotide

N.B. For all R_p s corresponding to T/C% (see Fig. 1), pairs with smaller increase in tumor volume is significantly larger at the .001 level for the group treated with CAP, except if otherwise specified.

* Not significant at the 0.05 level

first two compounds appear to be targeting aerobic glycolysis [1]. α -LA is known to inhibit the activity of pyruvate dehydrogenase kinase (PDK). This enzyme, which is upregulated in cancer cells that exhibit the aerobic glycolytic phenotype, phosphorylates pyruvate dehydrogenase (PDH), thus inhibiting its ability to convert pyruvate to acetyl CoA, the initial starting material for oxidative phosphorylation. The most likely effect of HCA is the inhibition of ATP citrate lyase (ACL), upregulated in cancer cells that exhibit the Warburg effect. ACL is a key enzyme involved in the biosynthesis of lipids, an absolute requirement for cellular proliferation, and its inhibition by HCA would be expected to significantly reduce tumor growth. Several studies have shown that OCT can inhibit the activity of mTOR, a key enzyme involved in cellular proliferation. Of particular note is the fact that mTOR activation has been shown to alter the isoform of pyruvate kinase (PK) from the M1 form that is present in most somatic cells to the M2 form, which is present in cancer cells as well as in embryonic cells and rapidly dividing somatic cells.

What role might CAP be playing? A large number of papers have been published demonstrating that CAP can inhibit cellular proliferation *in vitro* in a wide range of different types of cancer cell lines (ovary, pancreas, gastric, colon, breast, prostate, bladder, tongue as well as non-small lung cancer, leukemia, glioma, multiple myeloma cells, mitogen activated Jurkat T cells, the human nasopharyngeal carcinoma cell line; and the three human lymphoblastoid cell lines). Invariably, this inhibition of cellular proliferation has been through the induction of apoptosis. It is important to point out that the vast majority of investigators have demonstrated that capsaicin does not induce apoptosis in normal cells [see, for example, 17–19], although there is at least one report of capsaicin inducing apoptosis in MCF-10A cells, a normal breast epithelial cell line [20].

Given the large number of publications that have investigated the effect of CAP as a possible anti-carcinogenic compound *in vitro*, it is somewhat surprising that there are relatively few publications that have investigated the *in vivo* effects of CAP. Five articles described the CAP antitumor activity in different models: B16F0 melanoma, NB4 promyelocytic leukemia, PC-3 prostate cancer, U266 multiple myeloma, ASPC-1 pancreatic tumor, T24 bladder cancer and MB231 breast cancer cells [18, 19, 21–24]. Mean tumor reduction ranged from 15 % and up to 70 %. As can be seen, CAP had at least some effect in a fairly wide range of *in vivo* tumor models however, in all cases, the treatment led to a decrease in the progress of tumor growth with no instance of actual shrinkage being observed. Interestingly, in our hand, the combination of α -LA/HCA/OCT/CAP led to a marked reduction in tumor growth (ca. 95 %) with evident tumor shrinkage being observed.

In order to attempt to explain this result it is essential to provide some mechanistic hypotheses regarding the mode of action of CAP and put this information into the context of the mode of action of the other three compounds used. As it turns out, there are almost as many proposed modes of action for the anti-cancer activity of CAP as there are published papers. Nevertheless there are two distinct general mechanistic pathways that have been proposed that would appear to have more than ample support [25]. Before discussing these two pathways, however, it is important to discuss one mode of action for the anti-cancer activity of CAP that is almost certainly not involved. CAP has been used for more than 20 years to treat multiple types of pain stemming from pathological conditions that affect peripheral nerves (for example herpes zoster, diabetic neuropathy, amputation stump pain, and painful skin tumors) [26]. This effect is well-known to be mediated by TRPV1, one of six known receptors that are referred to as the vanilloid receptors. At one noticeable exception [27], there is virtually universal agreement among published studies that have investigated the anti-cancer effects of CAP that the mechanism of action of CAP is TRPV1-independent [19, 22, 28]. The two most reasonable pathways that would appear to explain the ability of CAP to induce apoptosis both relate to the fact that capsaicin is a Coenzyme Q (CoQ) antagonist and can thus inhibit both the plasma membrane NADH oxidoreductase (PMOR), which is a component of the plasma membrane electron transport system, and Complex I of the mitochondrial electron transport chain [29]. One group of researchers have hypothesized that the effect of CAP is directly on the mitochondria, whereas a second group of researchers has proposed that CAP-induced apoptosis is mediated by its effect on the plasma membrane electron transport system. Both of these hypotheses will now be briefly discussed.

The hypothesis that CAP could interact directly with the NADH oxidase system to induce apoptosis via a direct effect on the mitochondria has been sustained by several results obtained from COLO16 and SRB-12 clones lacking mitochondria (ρ^0 cells), as described by Hail [29, 30]. In 1995, Morré, et al. proposed that the ability of CAP to induce apoptosis in cancer cells was linked to its effect on a plasma membrane NADH oxidase [21]. The possibility that CAP induces apoptosis through its interaction with the plasma membrane oxidoreductase (PMOR) system had also been suggested at approximately the same time by Wolvertang et al. [31]. Moreover, other laboratories have also hypothesized the involvement of the capsaicin with the PMOR in order to explain its apoptotic-inducing effect on cancer cells [see, for example, 32–34].

In 1996, soluble and transmembranous NADH oxidase activities were identified by the Morré group; both of these proteins were strongly inhibited by CAP [35].

This report was followed by the finding that human sera from cancer patients, with a wide spectrum of different cancers, contained an NADH oxidase that was inhibited by CAP, whereas the NADH oxidase found in cancer-free individuals was not inhibited by CAP [36]. In 1998, Morré designated this protein as tNOX (tumor NADH oxidase, also designated as ENOX2) and distinguished it from the normal NADH oxidase, which was designated as CNOX [37]. The overexpression of tNOX in COS-1 cells, which do not express tNOX, resulted in a 10-fold increase in sensitivity to CAP and another tNOX inhibitor (-)-epigallocatechin-3-gallate (EGCG), thus linking the interaction of tNOX and CAP as an explanation for the anti-cancer activity of CAP [38].

As far as a link to aerobic glycolysis is concerned, the point was made that the inhibition of tNOX, an NADH oxidase, would lead to an increase in the NADH/NAD⁺ ratio, thus interfering with the glycolytic process [39]. This suggestion, although far from being established, is not illogical given that there are two molecules of NADH produced for every molecule of glucose metabolized to pyruvate, while the further conversion of pyruvate to lactate would regenerate the NAD⁺ consumed during the up-stream steps of glycolysis. Since alpha-LA clearly inhibits the conversion of pyruvate to lactate, and HCA may do so as well [1], CAP might indeed be more effective in inhibiting cellular proliferation in conjunction with the alpha-LA/HCA/OCT mixture. There is, however, one publication from De Luca et al. [40] that proposed that tNOX inhibitors, including CAP, could be causing apoptosis by elevation of ceramide, accompanied perhaps by the reduction of sphingosine-1-phosphate. Therefore, the combination of alpha-LA/HCA/OCT, which strongly inhibits aerobic glycolysis thereby forcing the cancer cell to increase its dependence on the mitochondria for the production of ATP, coupled with CAP, which causes the mitochondria to malfunction, might well be expected to be severely cytotoxic to cancer cells.

This discussion would be incomplete without mentioning that it has been invariably observed that treatment of cancer cells lead to generation of ROS, generally hydrogen peroxide (hydroperoxide) or the superoxide radical anion. Of significant interest is the fact that several investigators have examined the effect of N-acetyl cysteine (NAC) and have noted that this antioxidant can greatly attenuate or eliminate the effect of CAP on cellular proliferation and apoptosis [see, for example, 17, 18]. Treatment of CP-3 cells with NAC was even found to reverse the CAP-induced increase in ceramide levels [41]. Therefore, it appears clear that ROS are playing a key role in apoptosis induced by CAP, but there is currently limited understanding of what that role might be. It is interesting to note that one hypothesis as to

why most tumors shift their metabolic process to aerobic glycolysis is to protect the developing tumor from ROS generated by the mitochondria during oxidative phosphorylation. Although there is overwhelming evidence that ROS and/or other forms of oxidative stress are instrumental in some initiation pathways for cancer, there is accumulating evidence that at a later stage of tumor development, protection from ROS provides the tumor a competitive advantage (see, for example, 42 regarding the dual role of Nrf2 in cancer).

Despite the fact that there are many unknowns regarding the role that CAP is playing in the quaternary mixture that we tested, the results are sufficiently striking that they clearly require further work – both mechanistic and clinical studies. There is, however, one remaining issue that needs to be mentioned. A key condition of our approach to discovering novel cancer treatments targeted to cancer cell metabolism has been that the compounds we have selected are readily available and essentially non-toxic. CAP is clearly readily available in that it is a natural product. However, there may well be toxicity issues. A very recent article reviews epidemiological findings relating to the carcinogenicity of CAP. There are indeed studies that suggest that CAP may be carcinogenic, although these tend to be older studies, whereas newer studies do not support these findings [43]. A much older review has examined the relatively small number of in vivo toxicological studies. This review concluded that CAP has dual effects on chemically induced carcinogenesis, and that ingestion of large amounts can lead to a number of adverse events including necrosis and ulceration in addition to possible carcinogenesis [44]. A few of the papers cited in this Discussion have made some relevant comments regarding the possible toxicity of CAP. For example, Macho et al. [32] point out that CAP can release pro-inflammatory neuropeptides, such as substance P, which in turn can lead to mast cell degranulation. Chou et al. [45] cite papers presenting both epidemiological and in vivo data that suggest that CAP may be carcinogenic. On the other hand, Thoenissen et al. [19] point out that mice treated with CAP tolerated a dose of 5 mg/kg orally for 3 days/week with no undesirable side effects. This observation is consistent with our own results.

Nevertheless, there are sufficient concerns regarding the toxicology of CAP to ask the question if there are other compounds that might have the same effect with fewer potential side effects. There is evidence to suggest that four CAP analogues, designated as capsates, have the potential to substitute for CAP with respect to the induction of apoptosis in cancer cells [46]. In that two of these compounds are natural components of green peppers, they lack the inflammatory properties associated with CAP and, therefore, may be viable alternatives for CAP.

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Conflict of interest METABLOC is a trade mark of Biorébus. AG is an employee of Biorébus. The other authors declare that they have no competing interests.

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