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Stereocomplexes Formed From Select Oligomers of Polymer d-lactic Acid (PDLA) and l-lactate May Inhibit Growth of Cancer Cells and Help Diagnose Aggressive Cancers—Applications of the Warburg Effect

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Abstract: It is proposed that select oligomers of polymer d-lactic acid (PDLA) will form a stereocomplex with l-lactate in vivo, producing lactate deficiency in tumor cells. Those cancer cells that utilize transport of lactate to maintain electrical neutrality may cease to multiply or die because of lactate trapping, and those cancer cells that benefit from utilization of extracellular lactate may be impaired. Intracellular trapping of lactate produces a different physiology than inhibition of LDH because the cell loses the option of shuttling pyruvate to an alternative pathway to produce an anion. Conjugated with stains or fluorescent probes, PDLA oligomers may be an agent for the diagnosis of tissue lactate and possibly cell differentiation in biopsy specimens. Preliminary experimental evidence is presented confirming that PDLA in high concentrations is cytotoxic and that l-lactate forms a presumed stereocomplex with PDLA. Future work should be directed at isolation of biologically active oligomers of PDLA.

Keywords: lactate, Warburg, PDLA, cancer

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Hypotheses

1. It is hypothesized that oligomers of d-lactic acid (PDLA) can cross the cell wall, form templated stereocomplexes with l-lactate, and can inhibit cell growth of susceptible cancer cells through unchecked acidosis. These cells may not be able to expel hydrogen ions formed as a by product of glycolysis. It is also proposed that the PDLA oligomer-l-lactate complex would cause a decrease in extracellular lactate that would paralyze the lactate shuttle and possibly starve exterior cancer cells.
2. It is hypothesized that dextran or a similar polymer could be conjugated to PDLA oligomers, increasing their relative concentration providing amplification of #1.
3. It is hypothesized that PDLA oligomers could be conjugated to a stain or fluorescent probe providing rapid histological detection of lactate in tissue. This could be morphologically correlated by pathologists such that diagnostic criteria of “poorly, moderately, or well differentiated cancer” may be related to lactate concentration.

Introduction

In 1931 Otto Warburg was awarded the Nobel Prize in medicine for his pioneering work demonstrating glycolysis as the primary anaerobic glucose metabolism within cancer cells.¹ Unfortunately, to date, a universal therapy has not emerged from his work. Since that time it has become apparent that not all cancer cells utilize glycolysis to produce ATP, but some utilize oxidative phosphorylation (Krebs cycle) to generate energy.² More specifically, those cells in the interior of a tumor where the oxygen tension is lowest and the milieu more acidotic tend to utilize glycolysis while those cells in the periphery where oxygen tension is higher tend to utilize oxidative phosphorylation.³ Glycolysis generates protons that need to be transported out of the cell to avoid acid build up. Many of the hydrogen ions that are transported out of the cell are accompanied by lactate to maintain electrical neutrality. There are other mechanisms exclusive of lactate that generate anions to buffer the acid production of glycolysis. These include metabolism of pyruvate to bicarbonate by hydration of CO₂ catalyzed by various carbonic anhydrases and monocarboxylases.⁴ Decreasing the intracellular lactate concentration may

cause cell death from unchecked acidosis. The intracellular pHs of the interior and exterior tumor cells are similar but the extracellular fluid surrounding the inner cells is orders of magnitude more acidic.⁵ It has been shown that a lactate shuttle exists between inner and outer cells where lactate is converted to pyruvate in the outer cells and then metabolized by oxidative phosphorylation to produce ATP to sustain cancer cells.⁶

The explanation of which tumor cells utilize glycolysis is more complex than oxygen availability. Rapid rates of energy production require the expedient but inefficient fermentation of glucose if there are insufficient or functionally abnormal mitochondria to process glucose through oxidative phosphorylation.² In addition to the metabolic effects of lactate in glycolysis, lactate has also been shown to contribute to tumor cell invasion and increased cell motility.⁷

More recent investigations have tried to separate lactate production from the hypoxic effects on tumor growth by using genetic markers. These studies suggest lactic acidosis may be independent of oxygen tension and may be associated with a more favorable clinical outcome.⁸ If this concept is true then inactivation or trapping of lactate could worsen clinical outcome. In summary, just as Otto Warburg's hypothesis is not universal to all cancers, lactate physiology in tumors probably has variable effect on viability, progression and virulence in different cancers.

Brief Review of Clinical Trials of Chemotherapeutic Agents that Target Glucose Metabolism of Tumor Cells

These agents include Dichloroacetate (DCA), 2-deoxyglucose (2-DG) and Lonidamine.

DCA

DCA is a pyruvate dehydrogenase activator, inhibiting the activity of pyruvate dehydrogenase kinase (PDK), and thus promoting the conversion of pyruvate to Acetyl-CoA.⁹ Treatment with DCA shifts the fate of pyruvate from glycolysis to mitochondrial oxidative phosphorylation which is likely responsible for its tumoricidal activity. DCA is an inhibitor of all four isoforms of PDK.⁹ DCA has been used for the treatment of congenital and acquired lactic



acidosis with the most significant long term side effect of reversible peripheral neuropathy but sedation and elevation of hepatic transaminases have also been reported. The dose required to inhibit colorectal cancer cells in vivo is estimated to be 5–10 times that used in treatment of lactic acidosis.^{9,10} Potential serum therapeutic doses could be between 20 and 50 mmole/L.⁹ In a number of studies DCA has been shown to be a tumoricidal agent and has reduced lactate levels in growth media in a dose-dependent manner.¹¹ Some favorable results have been reported in five patients suffering from glioblastoma multiforme where DCA was used in combination with surgery, temozolomide and radiation.¹⁰ If PDLA is found to be tumoricidal, it is likely that PDLA will reduce lactate, by trapping, a different mechanism than proposed for DCA. Possibly confounding the results of future testing of metabolic cancer agents, in some instances, the in vitro testing of DCA has not correlated with tumoricidal in vivo studies.¹⁰

2-DG

2-DG is a glucose analog that is a competitive inhibitor of glucose. It is actively transported into cells and phosphorylated into 2-DC phosphate (2-DC-P) by hexokinase.¹¹ Further metabolism of 2-DC-P is not possible and thus glycolysis is limited. 2-DC also has additional effects on protein glycosylation separate from its effects on glycolysis and it may enhance radionuclide cytotoxicity.^{12–14} In vivo studies (in mice) have shown that 2-DG is synergistic with adriamycin and paclitaxel in transplanted human osteosarcoma and non small cell lung cancer.^{15,16} Combination chemotherapy with 2-DG has been trialed in patients with glioblastoma.¹⁶ Because 2-DG is an analog of glucose it produces actions and side effects in cancer and non cancer cells. 2-DG may have proconvulsant actions.¹⁷

Lonidamine

Lonidamine, a derivative of indazole-3-carboxylic acid is an inhibitor of oxygen consumption and suppresses glycolysis in cancer cells probably through hexokinase suppression.¹⁵ Its action is non selective, but synergistic with other chemotherapeutic agents and it has been used in clinical trials for breast, ovarian, lung and glial cancers.¹⁵ The major complication observed in more than a majority of patients was myalgia that required lowering the dose.¹⁸

Other metabolic chemotherapeutic agents in the development pipeline include: 3-bromopyruvate, an inhibitor of hexokinase, oxythiamine, an inhibitor of transketolase and pyruvate dehydrogenase, and 6-aminonicotinamide, an inhibitor of the pentose phosphate pathway.^{15,19} One major problem associated with present medications that target tumor metabolism is that they also target normal cells. If PDLA is tumoricidal, it is speculated that cells having mitochondria with the capacity to shuttle pyruvate may be less affected than the subset of cancer cells that exclusively utilize glycolysis.

An Explanation of the Unusual Stereocomplex Formation of Lactic Acid

The discovery of unique racemic crystallites or stereocomplex formation of lactic acid was reported by Ikada et al in 1987.²⁰ Normally, conglomerate racemic mixtures have a lower melting point than either enantiomer. However, when polymer l-lactic acid (PLLA) and PDLA are mixed together the melting point is approximately 230 degrees centigrade compared to their individual melting points of around 170 degrees centigrade. This reaction occurs in aqueous solution at room temperature without a catalyst.^{21,22}

Since stereocomplex formations occur with various blends of PDLA and PLLA, I speculated whether l-lactate (a monomer) would form a stereocomplex with small PDLA oligomers of less than 500 Daltons. If this were true, then there could be biologic applications of lactate trapping that could be used to target cancer cells. In order to prevent crystallized oligomers, a degree of polymerization between 7 and 11 monomer units may be optimum, but this may not be the case in vivo.²³ It has not been reported in the literature whether l-lactate will bind to PDLA oligomers, however hetero-stereocomplexes of PDLA with levo amino acids polymers have been prepared.²⁴ Also l-insulin has been reported to bind to PDLA and the clinical significance has not been reported.²⁵

Because the proof of this concept is so dependent upon the proposed stereocomplex formed by PDLA oligomers and l-lactate a preliminary experimental section is appropriate:



PDLA Interferes with l-lactate Measurement (Methods and Results)

Experiment #1

Forty mg of d-lactic acid (Sigma) was polymerized in a microwave oven according to the method of Pandey and Aswath.²⁶ The microwave polymerization was discontinued when the sample weighed 20 mg. Weight loss was from water during polymer esterification and dehydration. In normal saline, at a temperature of 37 degrees centigrade, a control solution of 1 ml of l-lactic acid (50 mg/L) (Sigma) and separately 1 ml of an experimental solution of 1 ml of 50 mg/L of l-lactic acid with 20 mg of PDLA were incubated for 2 hours. Measurement of l-lactate with L-Lactate Accuvin Test strips (Accuvin LLC, Napa, Ca), using stereospecific lactate dehydrogenase and a tetrazolium color indicator, measured less l-lactate (interference) in the experimental sample as evidenced by colorimetric determination. Experimental sample vs. control measured 10 mg/L vs. 50 mg/L of l-lactate. Other relevant tests using similar methodology and D-Lactate Accuvin Test strips are as follows:

1. Interference when PLLA (100 mg) was added to a 1 ml solution of 800 mg/L d-lactic acid.
2. No interference when 50 mg/L l-lactic acid solution was added to a 60 mg/L d-lactic acid solution (Table 1).

Conclusion of experiment #1: PDLA in normal saline solution decreases the measurement of l-lactate in normal saline solution and PLLA in normal saline solution decreases the measurement of d-lactate in normal saline solution.

Table 1. Interference reactions of PDLA and l-lactate and PLLA and d-lactate in saline.

| | <u>l-lactate</u> Test strip | <u>d-lactate</u> Test strip |
|---|--------------------------------|--------------------------------|
| l-lactic acid (0.05 mg) | 50 mg/L | |
| l-lactic acid (0.05 mg) + PDLA (20 mg) | 10 mg/L | |
| d-lactic acid (0.8 mg) | | 800 mg/L |
| d-lactic (0.8 mg) acid + PLLA (100 mg) | | not detected |
| d-lactic acid (0.05 mg) + l-lactic acid (0.06 mg) | 50 mg/L | |

Experiment #2

After approval from the director of the clinical laboratory and the chairman of the human studies committee of the Durham Veterans Affairs Medical Center and after vigorous exercise, 18 ml of blood was drawn from a human subject. The blood was collected in three vials each containing sodium fluoride/potassium oxalate. Tubes 1 and 3 were centrifuged at 3400 rpm for 15 minutes and the serum was frozen. To tube 2 was added and mixed 0.5 ml of a 1 ml normal saline solution containing a mixture of PDLA d-lactate oligomers with an initial weight of 100 mg of d-lactic acid that had been microwave polymerized with loss of 30 mg of water. Tube 2 was then centrifuged at 3400 rpm and the serum was frozen. Tubes 1–3 containing serum were frozen at –5 degrees centigrade for 12 hours and then defrosted in an incubator at 37 degrees centigrade for 3 hours prior to assay. The tubes were then placed on ice and processed in the laboratory.

Serum was processed using the Siemens Dimension Vista System Flex reagent cartridge at the Durham Veterans Affairs Medical Center. Reference values of l-lactate are in the range of 0.4–2.0 mmol/L (Table 2).

Conclusion of experiment #2: PDLA of unknown molecular weight oligomers reduces the measurement of l-lactate in blood samples without addition of catalyst or heat.

Proposed reaction



Experiment #3

To determine the ability of PDLA and PLLA to mediate cytotoxicity for malignant cells, freshly isolated human chronic lymphocytic leukemia (CLL) cells were cultured with the agents for 72 hours at 37 °C

Table 2. PDLA decreases measurement of l-lactate in blood.

| Tube | l-lacate conc. | PDLA | l-lactate conc. with dilution |
|------|----------------|--------|-------------------------------|
| 1 | 9.1 mmol/L | 0 | 9.1 mmol/L |
| 2 | 7.0 mmol/L | 0.5 ml | 7.58 mmol/L |
| 3 | 8.6 mmol/L | 0 | 8.6 mmol/L |

in 5% carbon dioxide/95% air using SFM™ tissue culture medium (Invitrogen, Carlsbad, CA). The cells were cultured in triplicate with eight 2-fold dilutions of the agents (from 2.000 to 0.016 mg/mL). Cytotoxicity was determined with an assay using the tetrazolium-based compound MTS (Promega, Madison, WI) [as has been done before].²⁷

Conclusion of experiment #3: The studies demonstrated cytotoxicity for the CLL cells, with the mean effective dose for 50% cytotoxic effect of 1.30 mg/mL for PDLA (1.93 and 0.66 mg/mL in patients A and B), and 2.14 mg/mL for PLLA (2.65 and 1.63 mg/mL in patients A and B). Thus, the PDLA was effective at a lower concentration than PLLA. However, a relatively high concentration was required for both of these for the anti-leukemia effects. If lactate trapping was the cause of cytotoxicity, probably PDLA contains a large number of inactive oligomers mixed with one or more active oligomer(s).

Stereocomplex model of PDLA and l-lactate

The beta helix strand formed by PDLA oligomers could template for l-lactate because the lactate anion is attracted to the carbonyl of the ester which carries a partial positive charge because of the inductive effects of the adjacent oxygen molecules. Hydrogen bonding could exist between the OH group of l-lactate and the ester oxygen of oligomers of PDLA. With these attractions, the methyl groups of PLDA and lactate are favorably oriented with minimal steric effects. This complex which has been more specifically named a homo-stereocomplex is probably distinct from the stereo PLDA~PLLA complex. At this time it is not possible to determine the minimum size oligomer of PDLA that would be needed to complex intracellular l-lactate, but the size could be less than 500 Daltons (Fig. 1).

Discussion of Experimental Section

The experimental section supports my hypothesis that PDLA oligomers form a stereocomplex with l-lactate. The reaction occurs spontaneously in both normal saline and plasma (ΔG negative and $K_{\text{equilibrium}}$ greater than 1). With the present data, it is not possible to predict any other variables of the reaction since the molecular weight of the poly-

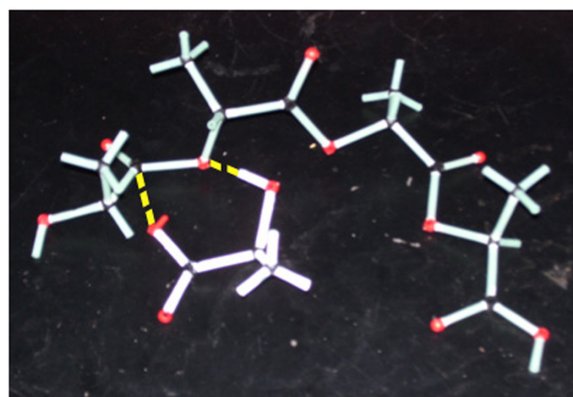


Figure 1. l-lactate (white) binds (yellow) to PDLA oligomers (green).

mer is an unknown. Molecular simulations could provide additional information about PDLA oligomers and l-lactate binding. Assuming that active size oligomer(s) are mixed in the microwave PDLA samples, separation of the polymer and data from isothermal titration calorimetry could determine which oligomer(s) are active as well as the reaction kinetics. Separation of the polymer to oligomers less than 500 Daltons (162, 234, 306, 378, 450 corresponding to 3x, 4x, 5x, or 6x d-lactate monomers respectively) may be the ideal weights for biologic application assuming that rapid plasma hydrolysis does not occur in vivo and multiple binding sites exist for l-lactate to complex with PDLA oligomers (Table 3).

Estimation of Three Compartment (Blood, Extracellular Tumor, Intracellular Tumor) PDLA Concentrations

The size, charge and lipophilic properties of the PDLA oligomers will mostly determine the likelihood that

Table 3. PDLA oligomer weights and hydrogen bond acceptors and donors.

| # of d-lactate monomers | Molecular weight | Hydrogen bond acceptors | Hydrogen bond donors |
|-------------------------|------------------|-------------------------|----------------------|
| 2 | 162 | 5 | 1-2 |
| 3 | 234 | 7 | 1-2 |
| 4 | 306 | 9 | 1-2 |
| 5 | 378 | 11 | 1-2 |
| 6 | 450 | 13 | 1-2 |



it will be available in the extracellular space to complex l-lactate. Using control values of $\text{pH}_e = 6.77$ (extracellular pH) and $\text{pH}_p = 7.4$ (plasma pH), assuming no active transport, the ion distribution of PDLA with a pK_a 3.86 would be approximately:⁵

$$\begin{aligned} \text{pH}_p &= 3.86 + \log \frac{[\text{PDLA}_p^-]}{[\text{PDLA}_p]} && \{\text{capillary}\} \\ 3.54 &= \log \frac{[\text{PDLA}_p^-]}{[\text{PDLA}_p]} && \{\text{capillary}\} \\ 3470 &= \frac{[\text{PDLA}_p^-]}{[\text{PDLA}_p]} && \{\text{capillary}\} \end{aligned}$$

$$\frac{[\text{PDLA}_p^-]}{[\text{PDLA}_p]} = 3470/814 = 4.26$$

Thus the approximate plasma concentration of the anion oligomer would be 4.26x that of the extracellular concentration.

The size, charge and lipophilic properties of the oligomer will mostly determine the likelihood that it will be available in the cytosol to complex l-lactate. However, the acid milieu of the extracellular compartment surrounding the tumor will aid in intracellular ion trapping of PDLA. Using control values of $\text{pH}_e = 6.77$ (extracellular pH) and $\text{pH}_i = 7.17$ (intracellular pH), assuming no active transport, the effects of intracellular ion trapping of PDLA with a pK_a 3.86 would be approximately:⁵

$$\begin{aligned} \text{pH}_e &= 3.86 + \log \frac{[\text{PDLA}_e^-]}{[\text{PDLA}_e]} && \{\text{cell wall}\} \\ 2.91 &= \log \frac{[\text{PDLA}_e^-]}{[\text{PDLA}_e]} && \{\text{cell wall}\} \\ 814 &= \frac{[\text{PDLA}_e^-]}{[\text{PDLA}_e]} && \{\text{cell wall}\} \end{aligned}$$

$$\frac{[\text{PDLA}_e^-]}{[\text{PDLA}_i^-]} = 814/2042 = 0.398$$

Thus the approximate intracellular concentration of the anion oligomer would be 0.398x that of the extracellular concentration. The $\frac{[\text{PDLA}_p^-]}{[\text{PDLA}_i^-]} = 1.7$ (plasma concentration to intracellular concentration). Since lactate concentration in some aggressive cancers may range between 10–12.9 micromoles/gm, ion trapping effects may have clinical importance.^{28,29}

Discussion

Evidence has been presented that PDLA of unknown molecular weight oligomers will spontaneously form a complex with l-lactate in aqueous solution and

plasma without a catalyst or addition of heat and there is some cytotoxic activity. Evidence has not been presented that PDLA is tumoricidal in vivo. The optimum oligomer size(s), lipophilic properties ($\log P$ or K_{ow}) and number of hydrogen bond donors or

$$\begin{aligned} \text{pH}_e &= 3.86 + \log \frac{[\text{PDLA}_e^-]}{[\text{PDLA}_e]} \\ 2.91 &= \log \frac{[\text{PDLA}_e^-]}{[\text{PDLA}_e]} \\ 814 &= \frac{[\text{PDLA}_e^-]}{[\text{PDLA}_e]} \end{aligned}$$

Antilog of equations
(Assuming
equilibrium across
the capillary)

acceptors needs to be trialed to assess whether PDLA oligomers of 2–4 monomers (possibly compatible with Lipinski's rule of 5 criteria) will complex intracellular l-lactate and be lead compounds.³⁰

How Could PDLA Oligomers be Useful Antitumor Agents?

It is possible that some cancers may respond to stereocomplex lactate trapping and others not. It is not clear from the experimental section what dose would be required to trap lactate in vivo and whether such a dose would have deleterious side effects. The average intra tumor lactate concentration reported

$$\begin{aligned} \text{pH}_i &= 3.86 + \log \frac{[\text{PDLA}_i^-]}{[\text{PDLA}_i]} \\ 3.31 &= \log \frac{[\text{PDLA}_i^-]}{[\text{PDLA}_i]} \\ 2042 &= \frac{[\text{PDLA}_i^-]}{[\text{PDLA}_i]} \end{aligned}$$

Antilog of equations
(Assuming
equilibrium across
the cell wall)

in aggressive head and neck tumors and cervical cancers has been reported as 12.9 micromoles/gm and 10 micromoles/gm respectively.^{28,29,31,32}

Without knowing the tissue density and location of the lactate (intra or extracellular) and kinetics of the stereocomplex reaction it would be difficult to estimate what concentration of PDLA oligomers would be potentially tumoricidal. However, multiple binding sites on oligomers less than 500 Daltons may be required for PDLA oligomers to be effective. Even if PDLA oligomers were not tumoricidal, it could lower tissue lactate concentration and produce less virulent tumors.



How Could PDLA be a Useful Diagnostic Agent?

With the addition of fluorescent or stain conjugates to PDLA, one may be able to detect lactate in biopsy specimens which have not been processed in usual fixatives. Because the stereocomplex reaction occurs in aqueous solution, it may be useful as an adjunct for frozen section diagnosis. Conjugated PDLA attached to dextran or another polymer may improve the assay.^{33,34} Present methods to measure tissue lactate levels are performed enzymatically with bioluminescent imaging.³⁵ This technique is complex and expensive.

Studies of Cancer Cells Lacking LDH Have Demonstrated that Lactic Acid is not the Major Mechanism Responsible for the Acid Environment of Tumors so Why Should Complexing L-lactate Increase Acidity and Inhibit Tumor Growth?³⁶

Complexing lactate does not produce the same physiology as inhibiting lactate formation by a substrate deficiency or inhibition of LDH. When LDH is inhibited the cell has the option of producing an anion through metabolism of pyruvate. This could include decarboxylation with formation of bicarbonate. When lactate is complexed the cancer cell loses the option to convert the pyruvate to an anion. Complexing may also drive the pyruvate-LDH-lactate reaction to the right since it would be unlikely that the complexed L-lactate would provide feedback inhibition for the reaction. Furthermore, changes in solubility or crystallinity can occur in some stereocomplex reactions that may also drive the reaction forward.²² Essential to the role of PDLA in therapy, it is not known under what conditions the PDLA~L-lactate will dissociate.

Could the PDLA~L-lactate Become a Substitute Anion for L-lactate and be Transported Out of the Cell?

Lactate is actively transported in and out of the cell by a monocarboxylate transporter. It would be unlikely that this transporter would recognize the stereocomplex. Passive diffusion of the stereocomplex could occur, but intracellular formation of the complex may

change the physical and chemical properties of the PDLA oligomer especially with respect to solubility.

Could Cancer Cells Substitute Another Anion for L-lactate?

Yes. This would be part of its adaptive process to survive.

What Other Mechanisms are Available to the Cancer Cell to Maintain Electric Balance and Restore its Normal Acidic Environment?

The cancer cell can restore its normal acidic environment through the vacuolar proton pump (V-ATPase), sodium/proton exchanger (NHE), bicarbonate transporters (BCT) and monocarboxylate transporters (MCT).^{37,38} However, with any of these systems there still needs to be an anion expelled to maintain electrical neutrality and the MCT and BCT systems still need a source for an anion which is likely pyruvate.

If PDLA Oligomers Complex L-Lactate, But is Not Specific for Cancer Cells, What are the Likely Consequences of Lactate Reduction?

This is presently unknown, however, low lactate levels are found in some patients with inherited lactate dehydrogenase (LDH) deficiencies. Patients with deficiencies of H subunits of LDH are usually asymptomatic while those with M subunit deficiencies may complain of easy fatigue and exertional myoglobinuria.³⁹⁻⁴¹ Lowering lactate by trapping with PDLA oligomers in normal cells will still allow alternative pathways for pyruvate such as conversion to Acetyl-CoA and decarboxylation. This could similarly be a problem in tumor cells that have mitochondria and capacity to redirect the pyruvate making it unlikely that PDLA would be a monotherapy.

What is the Bioavailability and Toxicity of PDLA?

PDLA has been shown to dissolve through enzymatic hydrolysis over short periods of time in solutions of phosphate buffered saline.⁴²⁻⁴⁵ Depending on the oligomer size and rate of PDLA ester hydrolysis it may be possible to design controlled released implanted PDLA for chemotherapy.⁴⁶⁻⁴⁸ The more



acidic extracellular pH of the tumor milieu may favor sequestration of the non-ionic form of the oligomer.

The LD 50 for PDLA has not been established. PDLA~PLLA is used in medical devices and as coatings on nanoparticles.⁴⁹ Ringer's lactate solution, containing a racemic mixture of lactate, is extensively used as an intravenous fluid in clinics and hospitals without complications from d-lactate. Rare cases of d-lactic acidosis have been describe in patients with short bowel syndromes and usually present with neurotoxicity and acidosis and most of these patients are successfully treated with carbohydrate restriction, hydration with non lactate fluids and sodium bicarbonate.^{50,51} If the etiology of the d-lactic acidosis is from bacterial fermentation in the gut, antibiotics may be prescribed. d-lactate levels greater than 3 mmole/L may be associated with symptoms.⁵¹ Since many factors such as degradation rate of PDLA oligomers by esterases, d-lactate metabolism, protein binding and dosing schedules may affect PDLA oligomer plasma concentration it is difficult to predict if d-lactate derived from PDLA will be toxic. D-lactate is a naturally occurring substance, ingested daily, and metabolized to a limited degree by humans.

Can PDLA Oligomers Selectively Bind to Lactate Associated with Cancer and Not to Normal Cells?

Probably not, since lactate is present in normal cells, interstitial fluid, plasma and cerebral spinal fluid. However, the extra and intra cellular pH gradients associate with some forms of cancer may preferentially increase concentrations of the oligomer. Furthermore, non cancer cells containing mitochondria can neutralize the proton load of glycolysis through alternative pathways such as oxidative phosphorylation that may not be available in many cancer cells.

How Could Pyruvate Levels Change With Formation of the PDLA~l-lactate Complex?

It is possible that decreased levels of pyruvate could destabilize hypoxia-inducible factor (HIF) as well as decrease the shunting of pyruvate for lipid and nucleic acid synthesis.¹⁹

Would PDLA Oligomers be a Monotherapy for Cancer?

Probably it would not. Other agents may need to target the acid lowering effects of BCI, MCT, V-ATPase and NHE. In addition, commonly used antineoplastic therapies most likely would need to be continued. The adaptation response of tumor cells to therapies has been mathematically modeled by Gatenby RA et al and timing of therapy with PDLA could be an important factor.⁷

What is the Likely Fate of the PDLA Oligomer~l-lactate complex?

The answer is pure speculation, but the fate may be different if there are solubility changes, cell death, or inhibition of cell growth.

What are Some Likely Flaws in this Concept?

1. For unknown reasons PDLA oligomers may not inhibit the in vitro growth of tumor cells, however agents that target metabolic pathways in cancer may be effective in vivo and not in vitro. In vivo testing may be required even if in vitro testing does not confirm tumoricidal activity.
2. More formal studies may show that the l-lactate~PDLA stereocomplex is non specific.
3. The quantities of PDLA and PLLA used in the experiments were exceptionally large and the dose required for complex formation may be toxic.
4. The l-lactate-PDLA oligomer stereocomplex may not occur in vivo because serum or intracellular esterases may hydrolyze PDLA oligomers making them ineffective.
5. Bioavailability or solubility prevents PDLA oligomers from being effective.
6. Oligomers of PDLA may not cross the cell membrane.
7. Cancer cells may adapt to intracellular l-lactate trapping and continue to grow in a more acidotic environment or another anion other than lactate is transported to the extracellular space to maintain electrical neutrality.

Conclusion

One needs to be cautious interpreting the concepts and experimental findings in this paper. I set out to investigate



two major concepts: 1) A stereocomplex forms between PDLA and l-lactate. 2) Trapping lactate will inhibit tumor cell growth. Neither concepts were conclusively proven, but the concepts were supported by experiments with inefficient (high) concentrations of PDLA that very likely contain biologically active oligomer(s) that trap lactate as well as inactive oligomer(s).

Optimum size oligomers of PDLA may have potential as antitumor and diagnostic agents forming stereocomplexes with l-lactate. Complexing intracellular lactate may produce acidosis differently than decreasing lactate by enzyme inhibition of LDH where the potential to convert pyruvate to an anion exists. Even if every concept that has been presented is proven, it is unlikely that PDLA oligomers would be a monotherapy for treatment of cancer, but rather an adjunct to present therapies. If concentrations of PDLA oligomers cannot be safely tumoricidal, PDLA oligomers could lower tissue lactate levels possibly decreasing tumor virulence. Complexing l-lactate with the smallest size oligomer having multiple binding sites, conforming to Lipinski's rules, balanced by the rate of plasma ester hydrolysis of PDLA oligomers, may produce the best therapeutic response. Stereocomplexes are unique formations, when discovered and appreciated, may translate into medical therapies. I hope this paper will enlighten those in medicine of the potential that select PDLA oligomers may have as a useful agent in cancer and other medical therapies when the need to trap lactate is diagnostic or therapeutic. Further work in the laboratory is enthusiastically encouraged to isolate, purify and conduct experiments to prove which oligomers of PDLA will be most beneficial.

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Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under

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