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REVIEW

Paracelsus Paradox and Drug Repurposing for Cancer

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ABSTRACT

Dose is one of the parameters that any pharmacologist seriously considers when studying the effects of a drug. If the necessary dose to achieve a desired pharmacological effect is in a toxic or very toxic range for human use, the drug will probably fall out from further research. The concentration that a drug can reach to its target organ or cell is a direct consequence of the administered dose and its pharmacodynamic properties. Basic researchers investigate at the cellular level or eventually with xenografts. They use different concentrations of the drug in order to determine its cellular effects. However, in many cases, these concentrations require doses that are in the toxic range or well beyond any clinically achievable level. Therefore, in these cases, research is in the realm of toxicology rather than therapeutics. This paper will show some examples about this exercise in futility which is time and resource consuming but that pullulates the pages of many prestigious journals. Many seasoned researchers seem to have forgotten the Paracelsus Paradox.

1. Introduction

If a cell whether normal or malignant is cultured in distilled water or even in tap water, the cell will die in a short time. Osmolarity in the first place and lack of nutrients will be the cause of death. Therefore, we can say that pure water is cytotoxic for both normal and malignant cells. Now let's change the circumstances, We give a glass of pure water to drink to a normal or sick individual and we shall discover that it is neither bad nor good. It has no effect and it is not cytotoxic. Finally in a new dramatic change of circumstances we administer the same pure water, but this time two liters via the trachea and the individual will be dead in a few minutes. In the movies, this last case would not be considered cytotoxicity but rather death by drowning according to the district attorney. In all three cases the substance used was plain water. In two out of three it was deadly, in one nothing happened.

What kind of a drug is plain water: cytotoxic, neutral or deadly? What changed in each occasion were the circumstances: applied to the cell, received per os, and finally a huge amount received by an unusual administration route.

This little game seems ridiculous, poorly planned and undoubtedly absurd. However, this is the way a large proportion of scientific papers work.

When we treat a malignant cell with 1,000 fold the highest level statins can achieve in circulation and we reach the conclusion that statins can be used as cytotoxic for tumors, aren't we playing the same game as above?

We coined a name for the game: PPP. It is not the Pentose Phosphate Pathway but the Potentiated Paracelsus Paradox.

2. Paracelsus Paradox

Sometime in the 1500s Paracelsus, the Swiss alche-

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mist and physician, established that any curative drug can at the same time be a poison, depending on the dose. We added the Potentiated by modifying one item in the Paracelsus Paradox: any curative drug is at the same time a poison, depending on the dose and the administration route.

Of course, nobody remembers Paracelsus anymore because he committed three sins:

He lived 500 years ago, did not have a Facebook page, and last but not the least, wrote in vulgata, sort of a bad Latin, today's equivalent of the most popular language in science, bad English.

PPP may be a good game for children, but does it have anything to do with the twenty-first century hard science?

If we are supposedly scientist let's examine the evidence before arriving at any conclusions.

3. Statins

We shall start by examining statins. There are many publications maintaining that statins have an anti-tumoral effect. Most of these papers are based on *in vitro* studies. However, these experiments may be misleading, because excessively high concentrations of statins were used, which cannot be reached in patients or if they are reached it would be at the expense of serious toxicity [1]. For example:

►Simvastatin concentration in plasma is in the mean range of 2.2-4.3 nM after an oral administration of 40 mg. The maximum concentration that can be achieved is 19-31 nM [2]. In a publication, *in vitro*, simvastatin at 20 μM induced breast cancer cell apoptosis [3]. This level is almost 1,000-times higher than the maximum that can be achieved in patients. The achievable plasma concentration of simvastatin was published in 2009 (probably there are prior determinations too). The researchers who used a 1,000 higher concentration published their results in 2012. This means that during all their experimental period they knew perfectly well that they were working in the realm of fantasy. A few questions are unavoidable:

Did the peer review process make no objections?

Have the 93 citations of this article in nine years taken for granted that simvastatin is a tumor apoptogenic drug without any further doubt?

Isn't using 1,000 fold the maximum achievable level of the drug like administering four liters of tap water through the trachea?

May be PPP is not a fantasy game but part of a game researchers like to play.

► Similarly, Hoque et al. [4] found that statins were able to induce apoptosis in prostate cancer cells when they were exposed to lovastatin at a concentration of 2 μM. However, a dose of 80 mg reaches a maximum concentration of 50 nM with an average of 9.4 nM [5]. The concentration used by Hoque et al. was 400-fold grater than what can be achieved in a patient. To this we must add that statin concentration in tissues, with the exception of the liver, are much lower than in serum [6]. Thus, most of these effects seen in vitro, are with concentrations many fold higher than those achievable in patients. Part of the game?

► Zhuang et al. [7] investigated the effects of lowering lipid rafts' cholesterol with simvastatin. They found that it reduced PI3K/Akt pathway signaling and induced apoptosis in LNCaP prostate cancer cells. Cholesterol replenishment activated Akt signaling and avoided apoptosis. Unfortunately, they used simvastatin concentrations of 20 μM, a level impossible to achieve in patients. This seminal finding clearly shows the mechanism by which cholesterol has an anti-tumorigenic behavior, but due to the excessively high simvastatin concentration it does not allow possible therapeutic conclusions. Simvastatin, as important as it is, is also a poison at very high concentrations, following Paracelsus principle: *sola dosis facit venenum.* Therefore, an unanswered question remains: can statins have the same effects at the concentrations they reach in human beings?

► Wong et al. showed that clinically achievable concentrations of statins had the ability to induce apoptosis in malignant cells through down-regulation of the anti-apoptotic protein bcl-2 [8,9]. The pro-apoptotic effect of statins was confirmed in many different tumor cell lines, including juvenile monomyelocytic leukemia [10], acute myeloid leukemia [11], myeloma [12], mesothelioma, pancreatic, colon [13], and prostate cancers [14], among others. However, when these experiments were analyzed in depth, the concentrations of lovastatin used were at the micromolar level while the achievable concentrations are in the nanomolar levels. This level is not feasible in the clinical setting. Thus, the tumor apoptotic effects of statins remain controversial [15].

► There are also experiments that take into account the achievable level of statins in blood. For example, Gordon et al. [16] administered oral simvastatin to LNCaP xenograft bearing castrated mice and the plasma level reached an average concentration of 3.29 nM without biochemical signs of toxicity. This level is found in humans taking 80 mg of the statin. The dose was effective to slowdown tumor growth and progression. Cholesterol *de novo* synthesis was also reduced. Can this be extrapolated to humans?

"Unfortunate" concentrations of statins raise doubts about the usefulness of some experiments and publications, but this does not preclude that in spite of these poorly planned experiments, the drug may have some anti-tumor effects.

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► Staying with statins let's go one step further. Many publications base the antitumoral action of statins on its ability to inhibit Ras farnesylation. Statins are inhibitors of HMG-CoA reductase, thus inhibitors of *de novo* cholesterol synthesis through the inhibition of mevalonate generation. Reducing cholesterol synthesis leads to a lower production of farnesylate which would decrease farnesylation of RAS and Rho GTPases and decrease its activation [17-20]. This mechanism is clearly anti-tumoral. However, it only works with very high concentrations of statins (50 μM). Surprisingly, Cho et al. [18] found that therapeutic levels of lovastatin, usually with a concentration range of 50 to 500 nanomoles (nM), not only did not decrease RAS activation, but increased it. This occurred as a consequence of phospholipase D activation. Therefore, therapeutic levels of lovastatin did not decrease RAS prenylation. In spite of this seminal finding, most of the publications repeat the mantra that statins decrease RAS signaling. This would only be true if highly toxic levels of statins were to be administered. This does not happen in patients treated with statins.

4. Metformin

Metformin is another paradigmatic drug to be repurposed almost for everything: aging, obesity, endometriosis, and fundamentally for cancer.

Since Evans et al. [19] in 2005, published their population-based statistical finding that metformin reduced the risk of cancer in diabetics, tons of papers have been published on this subject. Among half true and half erroneous concepts, some authors never really read the publication.

For example, an oncology book on repurposed drugs references the Evans manuscript saying that metformin has been used to treat cancer [20]. The Evans paper is a statistical study that shows a risk reduction of cancer in diabetics taking metformin for the treatment of diabetes, not for treating cancer. However, the authors say: "In 2005, MTF was used for breast cancer treatment."

These types of errors are not the focus of this manuscript. Let's analyze whether the research backing metformin as an "onco" drug is based on solid evidence.

What is the maximum non-toxic concentration of metformin?

Administering 500 mg of oral metformin to healthy volunteers the plasma concentration of metformin reached a maximum level of 1.42 μg/ml after two hours [21]. The usual daily dose does not exceed 3 g and the maximum approved total daily dose for diabetes mellitus is 2.5 g (35 mg/kg body weight) [22].

The concentration of metformin is high in the hepatic portal vein (approximately double than in cava vein), but after it emerges from the liver, the systemic plasma concentration is in the range of 10–40 μM in mice [23].

Reviewing the literature there is a wide range of metformin concentration in plasma which goes from 0.1 to 4 mg/l [24]. Therefore, we can assume 4 μg/ml to be the fair value. The molecular weight of metformin is 129.16 g/mol. We will use the maximal non-toxic concentration, 50 mg/l in humans (0.4 mM or 50μg/ml) as the upper limit of metformin that can be clinically reached with oral administration [25].

CONVERSION TABLE

4 μg/ml = 4 mg/liter = 0.004 g/l achievable plasma concentration with 500 mg metformin.

160 mg/liter was found in patients developing lactic acidosis with a mortality of 53% [26].

Molecular weight of metformin = 129.16 g/l

A solution with 1 Mol of metformin is a solution that contains 129.16 g/liter

1 mM of metformin is 129.16/1000 = 0.129 g/l = 129 mg/l = 129 μg/ml

Based on these data we analyzed some publications on metformin activity in cancer.

► Zhang et al. [27] studied the effect of 5 mM of metformin on CD133+ cells in colon cancer and arrived at the conclusion that "Inhibition of the proliferation of CD133+ cells may be a potential mechanism responsible for the association of metformin use with improved CRC outcomes in CRC patients with type 2 diabetes". 5 mM of metformin is the equivalent to 645 mg/l. This represents ~13-fold increase of what is a clinically achievable non-toxic concentration in plasma. To this we must add that the plasma concentration is always much higher than tumor concentration.

► Kim et al. [28] tested HeLa cells incubated with metformin. They found a decrease in proliferation starting at a concentration of 5 mM and from there on in a dose dependent manner. Again, the metformin concentrations were well beyond toxic levels.

► Jang et al. [29] used metformin for bladder cancer cells. A slight decrease of viability could be seen with metformin concentration of 3 mM. A clear drop in viability was only seen with concentrations between 9 and 12 mM. We are again in levels that are 20-fold higher than a tolerable dose.

► Griss et al. [30] showed that metformin decreased cancer cell proliferation *in vitro*, however they used concentrations between 2.5 and 10 mM.

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Table 1: Fenofibrate1's cytotoxicity on glioblastoma cells

Reference	Finding
Reiss et al., 2008 [47]	Fenofibrate sensitized glioblastoma cells to cisplatin.
Drukala et al. ,2010 [48]	Increased ROS potentiated fenofibrate's anti-glioblastoma effects.
Giordano et al., 2012 [49]	Fenofibrate induced apoptosis in glioblastoma cells.
Wilk et al., 2012 [50]	25 μM of fenofibrate induced glioblastoma cells G1 arrest with minimal apoptosis.. 50 μM achieved massive apoptosis. The mechanism was FOXO3 nuclear accumulation that induced BIM (an apoptotic protein) transcriptional activation.
Wilk et al., 2013 [51]	Anti-tumoral effects of fenofibrate on glioblastoma cells were found to be independent of PPARα agonism.
Binello et al., 2014 [52]	They found pro-apoptotic effects and CSCs migration inhibition with fenformin acting on glioblastoma cells.
Han et al., 2015 [53]	Fenofibrate inhibited NF-κB/RelA activation and by impeding its association with hypoxia inducible factor1 alpha (HIF1α), pyruvatekinase 2 is not expressed, thus favoring oxidative metabolism.
Grabacka et al. ,2016 [54]	Fenofibrate induced the production of ketone bodies in glioblastoma cells that cannot use them as an energy source, inducing growth arrest.
Kast et al., 2017 [55]	Fenofibrate decreased glioblastoma growth by decreasing/blocking glioblastoma induced granulocyte colony factor production.

The above mentioned publications were not selected, but randomly picked from Google Scholar under the search based on "metformin and cancer".

We are not saying here that metformin has no anti-cancer activity, as a matter of fact we believe exactly the opposite. The only objective is to show that many of the molecular mechanisms published as the fundamentals for metformin's anti-cancer effects, are based on concentrations that are absolutely impossible to achieve in patients. *In vitro* studies at poisonous levels, brings us back to Paracelsus concept and lack practical bedside application.

5. Capsaicin

It has been shown that capsaicin, present in hot peppers and chilli, has interesting anticancer effects. The most important seem to be promoting apoptosis in cancer but not in normal cells [31]. The anti-growth effects of capsaicin has been found in androgen- independent prostate cancer [32], squamous cell carcinoma KB cells [33], gastric [34], pancreatic [35], breast [36,37], colorectal [38,39], small cell lung cancer [40] cells among many others.

In spite of all this evidence favoring capsaicin, low concentrations have the ability to promote metastasis [41].

A research on capsaicin effects on renal cell carcinoma cells [42] showed that it had pro-apoptotic effects at an average concentration of 200 μM. One of the peer reviewers asked:

"Significant effect of Capsaicin was seen starting at the dose of 200 uM. What is the physiologically achievable concentration of capsaicin in Humans?"

Interestingly, the authors were unable to give a straight answer. They said: *"Lots of papers studied capsaicin at the concentration of 200 uM [1-3] (even 500uM [4]) in vitro cell models"*.

This means that they used 200 μM (equivalent to approximately 61 mg/ml. Capsaicin's molecular weight is 305 g/mol).) because other authors used the same concentration or even higher, but we still do not know if this concentration is clinically achievable. The only guide we found is that capsaicin in rats can reach 90 ng/ml in blood, 167 pg/mg in the lung and 3.4 pg/mg in the liver [43]. Suresh et al. [44] found an average concentration of 1.9 μg/ml after an hour of a high intake of capsaicin in rats. This level halved after another hour. In men, after a high intake of capsaicin containing food a level of 179 ng/ml was found [45].

Therefore, a concentration of 200 μM is far beyond the concentration found in rats and in humans. Prima facie, it seems very doubtful that 200 μM can be clinically achieved.

6. The Wrong Route

A different case is fenofibrate. This lipid lowering drug has clearly established antitumoral abilities [46], although not all the mechanisms are clearly known. Interestingly, there is a great deal of research going on regarding fenofibrate's activity on glioblastoma cells. We summarized them in Table 1.

From Table 1 the first conclusion would be that fenofibrate should be a first line treatment for glioblastoma. Furthermore, PPARα overexpression was found to be associated with a better prognosis in wild type glioblastoma [56] and precisely fenofibrate is a PPARα agonist.

However, there is a problem that some authors seem to forget: "fenofibrate does not cross the blood brain barrier and is quickly processed by blood and tissue esterases to form the PPARα agonist fenofibric acid, which is practically ineffective in triggering cancer cell death" [57,58]. Therefore until a practical approach to deliver fenofibrate into the brain tumor is found, all the research at the cellu-

lar level will remain in the laboratory. This we have called the administration route bias.

However, there are some authors that even recommended using oral fenofibrate (100 mg twice a day) in the clinical setting for glioblastoma as part of a multidrug scheme [59]. Unfortunately, they offer no proof of any benefit.

There is enough evidence to include fenofibrate in a clinical trial as a complementary drug for diverse cancers, except glioblastoma. As soon as the delivery system beyond the blood-brain-tumor barrier for fenofibrate becomes reality, this will change. At the present moment recommending the unmodified fenofibrate for glioblastoma in the clinical setting is at least a poor idea. If there are authors that pretend to use unmodified fenofibrate for glioblastoma, we wander if they pretend to drill a hole in the skull to smear 100 mg twice a day on the tumor. Otherwise, they will only lower triglycerides and we do not think that will change the course of events.

7. Silymarin

Is a compound obtained from milk thisthle (Sibylium marianum) that has been used for more than two thousand years for the treatment of diverse ailments. Nowadays, it is prescribed for the therapy of liver toxicity produced by ingestion of Amanita phalloides mushroom, and other chemicals and for non-alcoholic liver esteatosis. In the last twenty-five years silymarin and its main active principle, silybin, has been under scrutiny for the treatment of cancer [60].

SIL is not soluble in water and oral administration shows poor absorption in the alimentary tract (approximately 1 % in rats [61], however, other authors mention a higher absorption around 30%); it is mainly excreted in the bile. Toxicity is almost absent [62] and therefore high oral doses can be administered with negligible side effects.

In spite of this low absorption, according to Janiak et al. a plasma level of 500 mg/L (500 μg/ml) is achievable 90 minutes after oral administration of 200 mg/kg of silymarin in mice [63]. The elimination half-life is 6 hours.

240 mg of silybin were orally administered to six healthy volunteers and the following results were obtained: **maximum plasma concentration 0.34 ± 0.16 μg/ml** and time to maximum plasma concentration 1.32 ± 0.45 h. Absorption half life 0.17 ± 0.09 h, elimination half life 6.32 ± 3.94 [64].

Beckmann-Knopp et al. [65] found: *"Mean maximum plasma concentration after an oral dose of 700 mg silymarin, containing 254 mg of silibinin, is* ***317 ng/ml or 0.6 mM.*** *Accumulation in plasma during three daily medications is negligible. Plasma protein binding is reported to reach about 90–95%".*

Gatti et al. [66] found that free unconjugated silybin reached a maximum concentration of 141 ng/ml after 2.4 hours of feeding volunteers with 80 mg of a lipophilic silybin-phospatidylcholine complex (silipide). The level of conjugated silybin peaked after 3.8 hours reaching 255 ng/ml.

Another study on 6 healthy volunteers receiving 560 mg of silymarin attained concentrations ranging between 0.18 to 0.64 μg/ml [67].

In dogs [68], the silybin-phosphatidylcholine complex (SPC) showed increased concentration when compared with silymarin extract, however, the results show a low level in general:

SPC: 1,310 ± 880 ng/ml; silymarin: 383 ± 472 ng/ml.

Morazzoni et al. [69] found higher peak levels of silybin in the form of silipide when administered to rats: *"After oral silipide, silybin reached peak plasma levels within 2 h, with a* C_{max} *of 9.0±3.0 μg/ml for unconjugated drug and 93.4±16.7 μg/ml for total (free + unconjugated drug)".*

Concentration in humans (with a low dose) is far lower than what was found in rodents (with a high dose). The important issue to raise is that most of the experiments at cellular level that can be found in the literature use a concentration around 100 μg/ml. Even in the publication of Morazzoni the level of 100 μg/ml was not achieved and in any case it is a peak level that cannot be sustained. Therefore, is the experimental level achievable at the bedside?

We think that there is no evidence that it can be.

Oral administration of SIL in humans achieves nanogram levels but not micrograms. Furthermore, we should not extrapolate Morazzoni et al. findings in rats to humans.

Therefore, the evidence based on these high concentration experiments should be cautiously viewed.

8. Discussion

Five drugs, namely statins, metformin, capsaicin, fenofibrate,and silybin are considered in many papers as candidates to be repurposed for cancer treatment. The first three were used against cancer in *in vitro* and *in vivo* experiments. Many of the published articles in medical literature show that these drugs were essayed in concentrations that in most cases are impossible to achieve in patients.

The fourth drug, fenofibrate, was proposed at an oral dose of 100 mg twice a day for the treatment of glioblastoma when this drug is unable to cross the blood-brain barrier. Regarding silymarin and silybin the clinically achievable concentration may be enough for its antimigratory effects but are insufficient to induce apoptosis.

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Drug repurposing is a growing and useful research activity, however we cannot avoid asking how useful many of these publications are when they use these drugs under conditions that are impossible to attain in patients.

It is quite possible that silibyn serum concentration will be improved with the new pharmaceutical forms under research. Being non-toxic even at high doses, gives a good possibility to improve its anti-cancer scope. On the other hand, metformin represents an absolute barrier due to its toxicity. Achieving a higher plasma concentration will only increase the chances of lactic acidosis.

9. Conclusions

Some ongoing research on drug repurposing seems to belong more to the realm of Cesare Borgia than to reasonable and scientific molecular pharmacology. According to some not very reliable twisted stories, Borgia was a notorious master poisoner in sixteenth century Rome. If we poison the cell, it will surely die. Paracelsus cautioned us that we must use drugs within their therapeutic range. This seems to be ancient history for many twenty first-century researchers.

Some authors take for granted that what happens in the Petri dish, no matter the concentration of the drug, will also happen in the patients. The problem is that they forget that a drug to be effective has to reach its target.

Dose bias and administration route bias seem to represent a substantial part of present day cancer research.

Finally, we should ask how reliable peer review is, when all these supposedly reviewed but heavily biased articles pullulate in prestigious journals and in the web.

Paraphrasing Paracelsus: *Venenose portione vir necat, autem non sanat*.

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