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Backstory Commentary

Prodrugs: My Initial Exploration and Where It Led

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ABSTRACT

This review presents my early exploration in the area of prodrugs and specifically prodrugs of the anticonvulsant, phenytoin, also called diphenylhydantoin. My journey started in graduate school with an introduction to the prodrug concept and continued for much of my career as I remain fascinated by the topic/technique. I have also included some backstories that the reader might find noteworthy. Prodrug intervention is now recognized as one of the better tools for taking a challenging small molecule drug from un-developable to developable.

Introduction

The purpose of this look back on my early involvement in the field of prodrugs is to provide insight into how one person's contribution to a field can be influenced by luck, mentoring, and nurturing, and how that contribution can be enhanced by great students and collaborators who can execute and contribute to everyone's success.

In September 1968, I entered the graduate program in Pharmaceutical Chemistry at The University of Kansas (KU) chaired by the late Professor Takeru Higuchi, an icon in our field. How I ended up at KU and choosing, or being chosen, to study under Higuchi is another story. This retrospective is about my introduction to prodrugs.

My knowledge of prodrugs at the beginning of my graduate studies was very limited. I had recently completed a BPharm degree from the Victorian College of Pharmacy (VCP), now Monash University, in Melbourne, Australia in 1967/68, and then I had worked as a rural hospital pharmacist for nine months prior to entering KU. A number of drugs that I studied at VCP and dispensed in the pharmacy were in fact prodrugs, for example, the eye drops chloramphenicol hemi succinate[1] by Parke Davis, but the term "prodrug" *per se* was not familiar to me in September 1968.

About a month after entering KU, I met with my advisor, Professor Higuchi, about a research project. He handed me a one-page sheet of paper with about 5–6 chemical structures: succinimide and N-acyl derivatives of succinimide. He asked me to make the compounds and study them. "Who am I to question why?" was the thought on my mind as I walked out of the meeting. I spent the next month in the KU Science library and figured out how to synthesize the compounds but, in the back of my mind, the nagging question was always, "why?" A fellow student at the time, Ho Leung Fung, mentioned that "maybe these were intended as prodrugs." What was a prodrug?

Higuchi kept a collection of hard copies of PhD dissertations that he had chaired in the small departmental library. One was by a student of Higuchi's from his University of Wisconsin days (1946–87) by the name of Lewis (Lew) Dittert. After graduating from Wisconsin in the early1960s, Professor Dittert became one of the early contributors to the new field of biopharmaceutics and pharmacokinetics. That dissertation provided my first real introduction to prodrugs. Dittert had studied carbonate and carbamates, and his introduction included a short exposé on prodrugs.

I then met with Higuchi again, told him how I was going to make the compounds, and asked whether he minded if I worked with phthalimide instead of succinimide.[2] I then asked him the following: "is the reason for making and studying the compounds to disrupt hydrogen bonding interactions in the solid state of the imide and to see if the derivatives could then act as prodrugs?" After asking the question, I looked up and Professor Higuchi had this big smile on his face.

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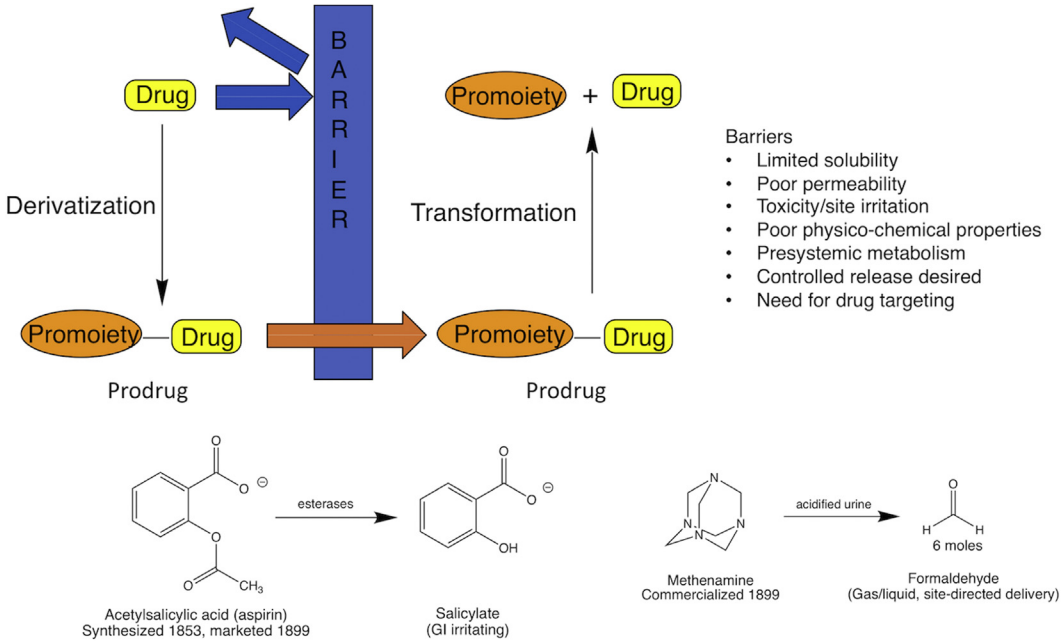

Fig. 1. Schematic of the prodrug concept along with some of the barriers for which prodrugs have been useful. Included are structures of two of the earliest prodrugs, acetylsalicyclic acid and methenamine.[4]

What are Prodrugs?

The term "pro-drug", with a hyphen, was first used by the chemist/scientist Professor Adrien Albert of Australian National University, Canberra, Australia, in a paper in Nature in 1958.[3] He also used the term "pro-agent". Albert is quoted as saying that some have argued that he should have called them pre-drugs.[4] To quote Albert when he explained that drug molecules must often cross at least one semi-permeable membrane to reach their site of action, "sometimes the substance (drug), as administered, is *only* a 'pro-drug' which has to be to be broken down to give the true drug. Examples of this kind are phenacetin, chloral hydrate …. " I did not like his use of the word "only" when describing pro-drugs and he dropped that "only" in subsequent papers and book editions. I suspect he received some reader feedback on the use of the term.

Albert first published his ground-breaking books in Medicinal Chemistry titled "Selective Toxicity, the Physico-Chemical Basis of Therapy" in 1951. In all, seven editions of Selective Toxicity were published.[4] The term pro-drug did not appear until the second edition published in 1960, a little after his 1958 Nature paper. I have jokingly said that one of the major contributions that I and Tony Sinkula made to science is the removal of the hyphen from Albert's "pro-drug." I met Albert at an IUPAC meeting in Paris in 1976, and we had a nice visit. He was a true gentleman.

Fig. 1 shows a schematic of the prodrug concept that has been used with some variations over the years. The challenge is that the delivery of the active drug is limited by some barrier. That barrier may be physico-chemical, such as poor aqueous solubility when an aqueous injectable form of the drug is needed or slow dissolution of the drug in the gastrointestinal tract (GIT) that limits its oral bioavailability. The barrier might also be a biological one where poor permeability across the cells lining the GIT or the blood brain barrier (BBB) could be due either to the high polarity of the drug or to its use as a substrate for an efflux mechanism. Other possible barriers are local irritation or toxicity. A listing of the common barriers is provided in Fig. 1 along with the chemical structures of two of the first prodrugs, acetylsalicylic acid (aspirin) and methenamine. Aspirin was first prepared around 1853 and eventually marketed by Bayer starting in 1899. The barrier in this case is that

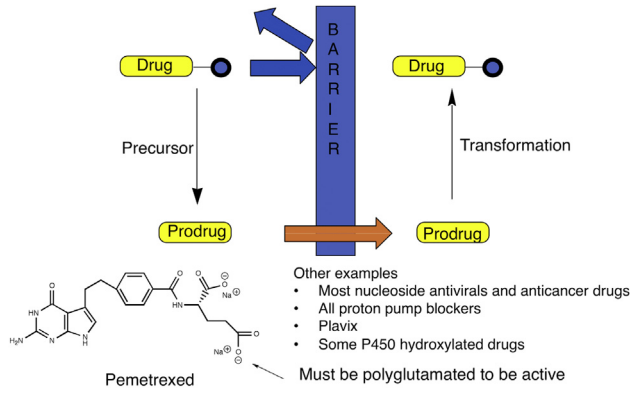

Fig. 2. Schematic of the precursor prodrug concept along with the structure of pemetrexed and other examples of precursor prodrugs.

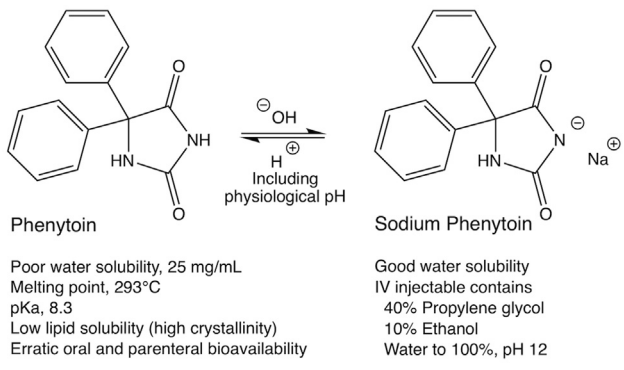

Fig. 3. Structures and some physico-chemical properties of phenytoin and sodium phenytoin.

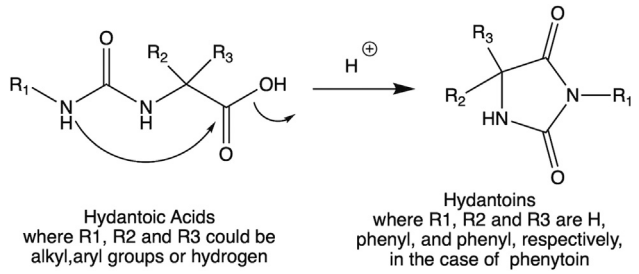

Fig. 4. Generalized structures of hydantoic acids and hydantoins with the scheme showing the cyclization of hydantoic acids to hydantoins under aqueous, acidic pH conditions.[13]

salicylic acid, the breakdown, active molecule when acetylsalicylic acid is used as an anti-inflammatory agent, is too irritating to the GI tract, while acetylsalicylic acid is less so. Albert claims that the first prodrug sold was actually methenamine, also called hexamine, by the Berlin company, Schering, also in 1899.[4] Methenamine is a prodrug of formaldehyde. Its main use is/was to treat bladder and kidney infections. For these types of prodrugs, the parent drug is derivatized to overcome the barrier, and once it crosses the barrier, the prodrug is designed to break down, usually via a metabolic event, releasing the active drug. In the case of methenamine, the bioreversion is triggered by a non-enzymatic event, the acidification of urine pH. Easy to say now, but quite an accomplishment to achieve over 100 years ago!

Fig. 2 shows a second prodrug concept, referred to as precursor prodrugs, or bioprecursor prodrugs, terms that I like. As before, the parent drug has a barrier to its delivery, but in this case, a precursor is used that can be converted to the active drug through a metabolic activation process found in the body. The example used in Fig. 2 is the mono-glutamate anticancer drug, pemetrexed, which is polyglutamated intracellularly to its active form using the same mechanism by which folic acid is polyglutamated.[5] Most older nucleoside antiviral and anticancer drugs are also precursor prodrugs in that their triphosphate nucleotides are the active molecules, not the nucleosides. The triphosphate active molecules are incapable of entering cells and are also rapidly dephosphorylated by phosphatases.

And Dissertation Projects Evolve

Within a year or so of starting my original project, I was introduced by Higuchi to Professor Sidney (Sid) Riegelman, one of Higuchi's early Wisconsin graduates who was then a professor at the University of California at San Francisco (UCSF). Riegelman, an early pioneer in pharmacokinetics in the USA, was on the scientific advisory board of Alza and had just attended a Scientific Advisory Board meeting in Lawrence, KS.

Riegelman and his students had been studying the intravenous (IV) pharmacokinetics in dogs of phenytoin, a hydantoin with

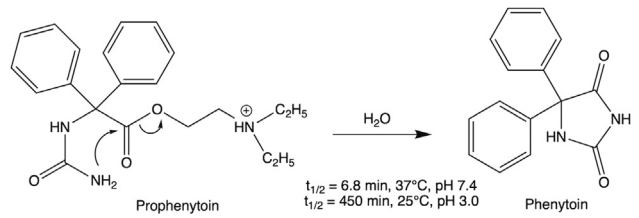

Fig. 5. Structure of prophenytoin and phenytoin with the scheme showing the cyclization of prophenytoin to phenytoin under two pH and temperature conditions.[14–16]

Table 1
Some Physico-Chemical Properties of Salt Forms of Prophenytoin from Stella et al.[16]

Salt	Mol. Weight	% Phenytoin Equivalents	Solubility, 25 °C
Nitrate	432	58.3	22 mg/mL
Hydrochloride	405.5	62.1	23 mg/mL
Salicylate	507	49.7	8 mg/mL
Sulfate	837.01	60.28	301 mg/mL

similar physico-chemical properties to imides. It was then sold as sodium phenytoin under the commercial name Dilantin® by Parke Davis to treat epilepsy. The structure of phenytoin and sodium phenytoin, some of their physico-chemical properties, and the injectable formulation of Dilantin® are provided in Fig. 3.

Riegelman mentioned in passing that he had noted a number of deaths in dogs from intravenous (IV) sodium phenytoin. He said that on autopsy, the presence of a white material in the lungs of the animals was consistent with neutral, poorly soluble, phenytoin precipitating and getting trapped in the lungs. He attributed the deaths to lung emboli. Toxicity and fatalities caused by too rapid an injection of IV Dilantin had also been noted in humans.[6–11] These were attributed to the same potential for precipitation as well as to the cardiac depressant action of phenytoin and propylene glycol and perhaps ethanol used in the formulation. Riegelman suggested the need for a safer form of phenytoin than the current IV Dilantin product and potentially one that could also be administered by the intramuscular route (IM). Thus was born the project to identify a safe prodrug of phenytoin without the safety limitations of the IV Dilantin® product.

Back to the Library!

Phenytoin is 5,5-diphenylhydantoin, a very effective drug to treat grand mal seizures, a severe form of epilepsy, and a number of other conditions. As seen in Fig. 3, it is sparingly water soluble, and the only way that a concentration of 50 mg/mL of sodium phenytoin solution for IV injection can be achieved is by forming the sodium salt by deprotonating the N_3H group with a pKa of 8.3 to form sodium phenytoin. Thus, the pH of the solution has to be around pH 11.5 or higher to reach a 50 mg/mL solution of sodium phenytoin. To further enhance solubility, a co-solvent solution of 40% propylene glycol and 10% ethanol with the balance as water was also needed. The poor solubility of phenytoin is due in part to its hydrophobicity, but more importantly to its highly crystalline nature that is due to strong hydrogen bonding in the solid state.[12]

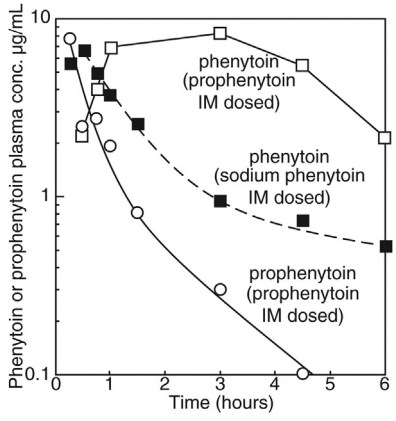

Fig. 6. Plasma levels of phenytoin and prophenytoin after IM administration of 45 mg/kg phenytoin equivalents of sodium phenytoin or prophenytoin to rats, Galzko et al.[15] Reproduced with permission.

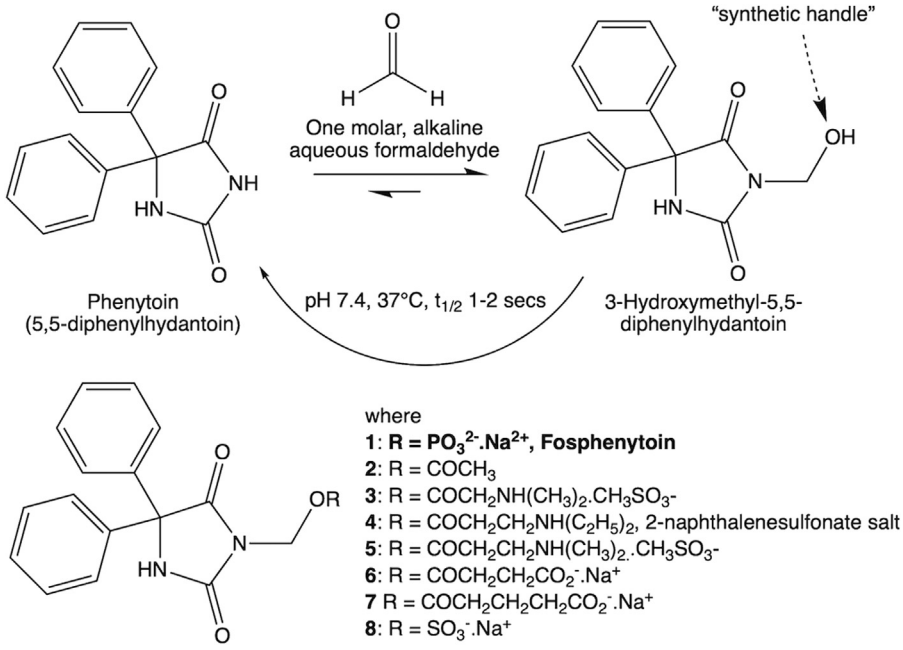

Fig. 7. Scheme showing the formation of 3-hydroxymethyl-phenytoin (3-hydroxymethyl-5,5-diphenylhydantoin) from the reversible reaction of phenytoin in aqueous formaldehyde solution and its rapid reversion in the absence of formaldehyde. Also illustrated is the structure of fosphenytoin (1) and various other phenytoin prodrugs 2–8.[30,34,38,40–42]

The high crystal packing energy can be seen by its high melting point (MP) of 293–295 °C.

The literature at the time did not provide any major clues or examples of earlier phenytoin derivatives or prodrug attempts except for some N_3-acyl and N_3-carbonate derivatives not unlike those suggested by Higuchi in my initial assignment for succinimide, but such derivatives had very poor water solubility and limited chemical stability.

As I was reading about the degradation pathways of hydantoins as well as their synthetic pathways, a thought occurred to me: hydantoins can hydrolyze (very slowly) to a ring-opened form called hydantoic acids, which under some conditions can cyclize to the hydantoin as illustrated in Fig. 4.

Hydantoic Acid and Hydantoic Acid Esters as Prodrugs of Hydantoins

Could hydantoic acids be prodrugs of hydantoins? In a paper published in 1973, we explored that potential and concluded the following: (1) yes, hydantoic acids in their carboxylic salt form are ionizable and so probably water soluble around physiological pH values; (2) yes, hydantoic acids can cyclize fairly rapidly but only at acidic pH values; and (3) no, the cyclization is too slow to consider them as prodrugs of hydantoins.[13]

What about hydantoic acid esters? The cyclization reaction to form hydantoins is more facile because an alcohol is a better leaving group; however, would it be fast enough? A simple, neutral ester would still likely display poor water solubility. Therefore, to achieve an aqueous solution of 50 mg/mL sodium phenytoin equivalents, one would have to build in a polar (and preferably ionizable) group capable of forming a salt.

The chemistry of cyclization of hydantoic acids and their esters to their respective hydantoins was a significant part of my PhD dissertation, which I defended in the late summer of 1971. The cyclization of various hydantoic acid esters to their anti-convulsant hydantoins was published in 1973.[14] In collaboration with researchers at Alza, and later Interx, a water-soluble prodrug of phenytoin (prophenytoin, see Fig. 5) was prepared, and its synthesis, physico-chemical properties, activity and pharmacokinetics after oral and parenteral administration were published in 1975 in one of the first, reasonably comprehensive books on the subject of prodrugs.[15–17] There were a number of earlier reviews such as those by Harper[18,19] and Kupchan[20] on prodrugs, although Harper preferred to use the term "drug latentiation" to "prodrugs". The two reviews by Sinkula and Yalkowsky[21] and Sinkula[22] are also historically very important.

Fig. 5 illustrates the half-lives ($t_{1/2}$) for prophenytoin conversion to phenytoin under simulated physiological conditions (pH 7.4 and 37 °C) and simulated short-term storage conditions (pH 3 and 25 °C).[16]

What Worked with Prophenytoin?

Prophenytoin exhibited excellent solubility as shown in Table 1.[16] Several salts were isolated and characterized but only to a limited extent. As with any acidic salt of any amine, solubility can vary dramatically and in unpredictable ways due to our inability to predict crystal packing. Hydrochloride salts are often preferred; however, in the case of prophenytoin, the hydrochloride salt had a solubility of only 23 mg/mL, which is equivalent to 14.3 mg/mL phenytoin or 15.5 mg/mL sodium phenytoin. Somewhat surprisingly, the hemi-sulfate salt showed a solubility of 301 mg/mL, which translated to >150 mg/mL phenytoin equivalents, and the solution had a pH of about 3.3. The salt was not further characterized as to whether it was crystalline or amorphous.

Prophenytoin showed excellent anticonvulsant activity.[15] It was further evaluated in studies carried out at the Research Laboratories at Parke Davis in Ann Arbor, MI. Glazko et al. performed extensive studies after intramuscular (IM) and oral administration in rats and after IV administration in dogs.[15] In all cases, fairly quantitative and reasonably complete delivery of phenytoin was achieved after IV dosing, and prophenytoin showed superior levels of phenytoin after IM administration compared to sodium phenytoin.[15] Oral dosing levels were also excellent. The data from the IM study in rats is

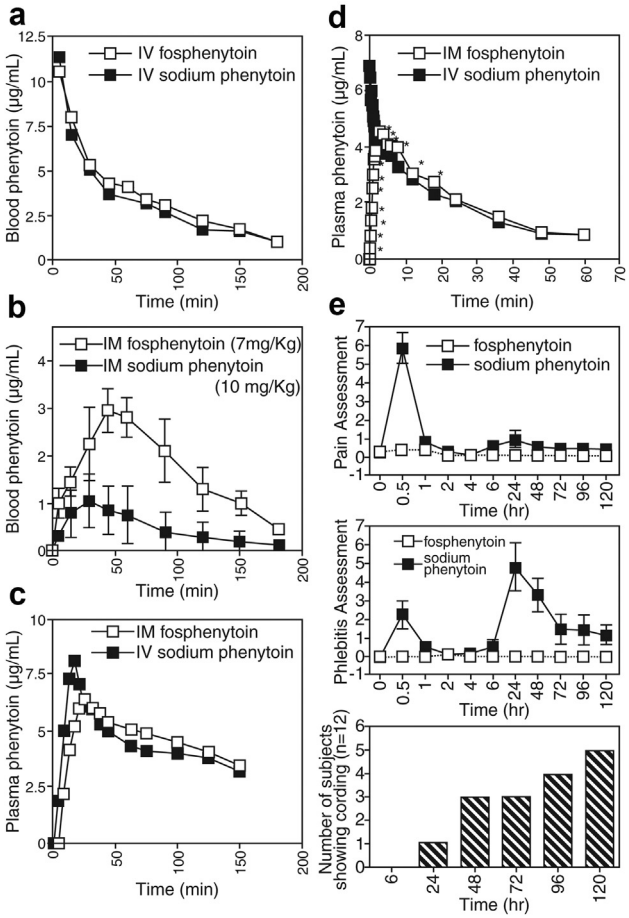

Fig. 8. A composite figure comparing fosphenytoin to sodium phenytoin from various studies. All figures reproduced here with permission. (a) Data comparing the whole blood concentrations versus time of phenytoin from equimolar 10 mg/kg IV sodium phenytoin and fosphenytoin administered to the same rat.[38] (b) Data comparing the whole blood concentrations (±SD) versus time of phenytoin from 10 mg/kg IM sodium phenytoin and 7 mg/kg IM (phenytoin equivalents) fosphenytoin administered to three rats.[38] (c) Plasma concentrations in 12 patients who received 30 min infusions of 250 mg equimolar doses of sodium phenytoin or fosphenytoin.[45] (d) Plasma concentrations in 12 patients who received a 10 min IV infusion of 250 mg sodium phenytoin and IM fosphenytoin. The asterisks indicate where significant differences were seen.[50] (e) Safety data comparing pain (top plot) and phlebitis (middle plot) after equimolar doses (250 mg phenytoin) administered in a 30 min IV infusion of sodium phenytoin and fosphenytoin in 12 subjects. The bottom plot shows the incidence of cording after the sodium phenytoin infusion. No cording was seen after fosphenytoin infusion.[49]

reproduced in Fig. 6. The inferior levels of plasma phenytoin obtained after IM administration of sodium phenytoin result from precipitation of phenytoin at the injection site, and this observation is consistent with the findings in human subjects.[23–26]

What did NOT Work Well with Prophenytoin?

Chemical stability was limiting for prophenytoin.[16] As was the issue with sodium phenytoin injectable if the pH of the solution were to drop, the poorly soluble phenytoin would precipitate from prophenytoin solutions upon storage. The chemical conversion of prophenytoin to phenytoin is the result of the intramolecular attack of the terminal ureido nitrogen at the ester functionality. In solution, this reaction can only be slowed by lowering the temperature and adjusting the pH to low values, but it cannot be stopped. Adequate short-term aqueous stability was only achievable at around pH 3–3.5 where precipitation of the formed phenytoin was

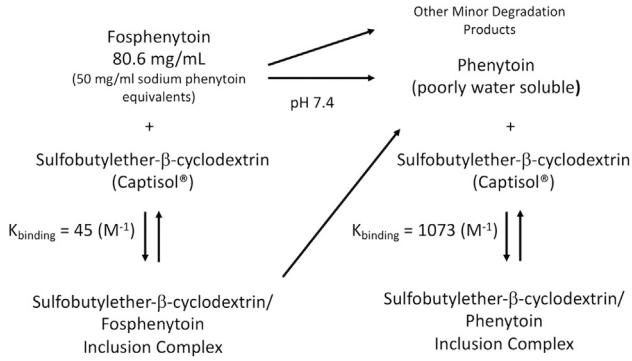

Fig. 9. Schematic of the competitive interaction and degradation of fosphenytoin to phenytoin in the presence of sulfobutylether-b-cyclodextrin (Captisol®) at pH 7.4 and 25 °C. From Narisawa and Stella.[57]

seen after about 1–2 h.[16] For longer-term storage, a freeze-dried formulation was needed to achieve an adequate shelf-life.[15,16]

Further development of this prodrug as a potential clinical candidate was terminated because of its significant cardiovascular activity, which followed the pharmacokinetic time profile of the prodrug. When designing prodrugs, one wants the prodrug itself to be safe, non-toxic and inactive. One element of safety is for the prodrug to have no inherent activity or – if there is some activity – it should at least be complementary to that of the final formed active drug, in this case, the parent phenytoin. In this case, the cardiovascular activity seen with prophenytoin was such that the prodrug was considered unsafe.

Lessons Learned from this First Experience with Prodrugs

Several key lessons learned from the prophenytoin experience impacted much of my career in prodrugs:

- A patent could be granted for a novel compound: my first patent, “Esters of hydantoic acid”, was issued in 1974.[27]
- Different salts of ionizable drugs can have dramatic differences in solubility.
- Many prodrugs can be chemically quite unstable, which can present additional hurdles to prodrug formulation/development.
- While solving one problem, others may arise. Yes, prodrugs are new chemical entities (NCE) and each new NCE has its own potential activity/toxicity.
- In the late 1960s, the new field of pharmacokinetics was critical to the evaluation of the effectiveness of prodrugs. I resolved to learn all I could.
- Prodrugs are a novel problem-solving technique to be explored further even if the first adventure was less than 100% successful.

After Prophenytoin

In the fall of 1971, I assumed an assistant professor position in the College of Pharmacy at the University of Illinois in Chicago. There I continued some research on prodrugs unrelated to phenytoin prodrugs for parenteral use. I returned to the University of Kansas as an asistant professor in the summer of 1973. Sometime in 1973/74, Professor Higuchi, now my Chairman, was asked to organize a symposium on prodrugs by the Medicinal Chemistry division of the American Chemical Society to be held in Atlantic City in the Fall of 1974, and he asked me to help. This involved not only lining up speakers for the symposium but also writing chapters and

Table 2
Rate of Formation of Phenytoin at pH 7.4 and 25 °C from 80.8 mg/mL Fosphenytoin (Equivalent to 50 mg/mL sodium phenytoin) in the Presence and Absence of 60 mM Sulfobuytlether-β-Cyclodextrin (Captisol®) and the Projected Shelf-Life Based on the Limiting Solubility of Phenytoin or 230 μg/mL Phenytoin Under the Same Conditions.

Cyclodextrin (Captisol®) Concentration	Phenytoin Formation Rate (μg/mL/day)	Phenytoin Solubility (μg/mL)	Projected Shelf-Life (years)[a]	Projected Shelf-Life (years)[b]
0	0.160	49.8[c]	0.9	0.9
60 mM	0.133	450.8	9.3	4.7

[a] Projected shelf-life based on the time to exceed the solubility of phenytoin under the stated conditions.
[b] Projected shelf-life based on the time to exceed 0.5% phenytoin formation.
[c] The aqueous solubility of phenytoin is enhanced by the presence of 80.8 mg/mL fosphenytoin from about 25 μg/mL to 49.8 μg/mL.
From Narisawa and Stella.[57]

editing a book on prodrugs. This was a seminal moment as it introduced me to Dr. Anthony (Tony) Sinkula from the Upjohn company and Dr. Anthony (Tony) Glazko from Parke Davis. At the time, Upjohn and Parke Davis were two US pharmaceutical companies that had embraced the use of prodrugs to overcome drug formulation and delivery issues for both NCEs and established drugs with various limitations.

In preparing my talk and writing the opening chapter, a review of prodrugs, I was exposed to the plethora of literature on prodrugs, including many successful commercial products as well as broader experimental prodrug concepts. The friendship and mentoring of Tony Sinkula, who wrote a chapter on prodrugs of antibiotics for the book, was further critical to my development. His published review article with Yalkowsky in early 1975 on prodrugs was a *tour de force* at that time.[21]

In 1975, our edited book "Pro-drugs as Novel Drug Delivery Systems" was published.[17] It not only helped me get tenure in 1976 at the University of Kansas but launched a life-time interest in the study of prodrugs.

Thank You, Professor Ian Pitman and Padam Bansal

Sometimes, one has the privilege to stand on the shoulders of others. In the mid-1970s, a colleague at the time, Professor Ian Pitman, and a graduate student of his and Higuchi's, Padam Bansal, were studying the reversible formation of hydroxymethyl compounds when various nitrogen heterocycles were exposed to increasing concentrations of formaldehyde in water. Their interest lay in studying how the aqueous solubility of the nitrogen heterocycles was affected by hydroxymethylation.[28,29] They showed that these hydroxymethyl derivatives can readily break down to release the parent heterocycle in the absence of added formaldehyde. Subsequent kinetic studies by us[30] and Bundgaard and Johansen[31] confirmed their observations and provided valuable insight into the speed of the dehydroxymethylation based on solution pH, temperature and the structure of the amide or heterocycle that was hydoxymethylated.

I realized that the work of Pitman, Higuchi and Bansal could be taken further: a *light bulb moment*, one might say. Could phenytoin be reversibly hydroxymethylated such that the formed hydroxy group providing a synthetic handle for further derivatization (Fig. 7)? An idea was born that had implications for phenytoin and many other drugs over the next 40+ years, although others had pioneered the idea of formaldehyde-spaced promoieties with the esterification of carboxylic groups such as with bacampicillin and other β-lactam antibiotics.[32]

Fosphenytoin

Around 1975/76, I hired a young military veteran by the name of Steve Schuler as an undergraduate hourly research assistant. He was a prepharmacy student. We also worked with a colleague at Interx, Dr. Ken Sloan. With Steve and Ken's help, we were able to make large and pure quantities of 3-hydroxymethyl-5,5-diphenylhydantoin and then a number of its esters (see Fig. 7.) The one derivative that eluded our initial synthesis efforts was the 3-phosphoryloxymethyl derivative, later known as fosphenytoin. However, we were eventually able to accomplish its synthesis with excellent purity and yields.[30,33]

Why the interest in a phosphate ester, Structure **1** in Fig. 7? Acyl derivatives, such as Structures **2**–**7** (Fig. 7), tend to have (and did have) limited chemically stability in aqueous solution.[34] However, good chemical stability is essential for injectables, especially when the degradation product, phenytoin, is highly water insoluble and likely to form particulates on standing. The other non-acyl derivative, the sulfate ester, **8**, did not biorevert to phenytoin on IV dosing to rats.[35]

When I was literature searching for the prodrug 1975 review paper and book, I became aware of phosphate esters of steroids as water-soluble prodrugs for parenteral administration and even for oral administration.[36] They are quite chemically stable, especially around physiological pH values, even to the extent that ready-to-use formulations are sometimes possible.[37,38] That is, a freeze-dried formulation is often not necessary to effect long-term storage stability of phosphate esters. While chemically quite stable, they most often are excellent substrates for alkaline phosphatases.[39] I had a note in a laboratory book, now lost, where I drew the structure of what became fosphenytoin (**1**) and wrote, "if I can make it, it will work."

The acyl esters proved somewhat useful and interesting, and much of the work was part of the dissertation of a PhD student, Salish Varia. However, fosphenytoin was the most effective of all the prodrugs studied. While the initial synthesis of fosphenytoin proved challenging to both us and others, later on, better, cleaner and simpler synthetic pathways were developed.[33] The synthesis, physicochemical properties and formulation of fosphenytoin were subsequently published by us along with a limited number of animal studies.[30,34,38,40]

The key initial findings from our laboratory for fosphenytoin were as follows:

- It is quite chemically stable with a pH of maximum stability around physiological pH 7.5–8.[34]
- It is rapidly and quantitively converted to phenytoin in rats and dogs after IV administration with half-lives on the order of minutes.[38,40]
- Unlike sodium phenytoin injectable, fosphenytoin provides significantly elevated phenytoin levels after IM administration to rats with no apparent tissue damage.[38]
- It provides excellent phenytoin levels even after oral administration to rats and dogs.[38,40]
- It has no apparent intrinsic activity of its own within the limits of the experiments performed, and any effects noted could be attributed to the formed phenytoin.[30]
- The original plea of professor Riegelman was achieved, albeit a number of years later, but was it good enough to commercialize?

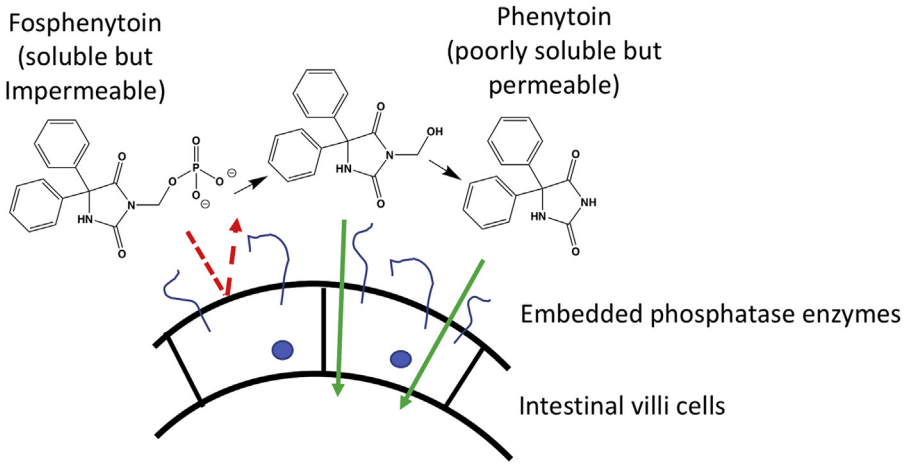

Fig. 10. Schematic showing how phosphate ester prodrugs, in this case fosphenytoin, can be used for effective oral delivery of permeable but poorly water-soluble drugs such as phenytoin. This schematic is similar to those employed by others.[75–77]

To Market, To Market!

Patents that included claims for fosphenytoin were filed in 1978 and issued in 1979 and 1981, and they were assigned to Interx, who had funded the research.[41,42] Around 1981, Interx was purchased by Merck to allow Professor Higuchi to study and develop device-oriented drug delivery systems for which he was otherwise limited due to a 10 year "no compete" clause when Higuchi broke with Alza and started Interx around 1971. Merck did not appreciate, at the time, that Interx also owned the patent to fosphenytoin, a point that I discussed with Higuchi and brought up with Merck management.

I negotiated an agreement with Merck for a modest increase in royalty and permission to try to find a company interested in taking on the development of fosphenytoin. The logical developer would have been Parke Davis, the originator of the Dilantin line of phenytoin products, but I was unable to make any headway with them as they were in the middle of merger discussions with Warner Lambert at the time. I was eventually able to get a small Chicago-based company, American Critical Care, interested, and a licensing contract was signed. The first 100 g or so of fosphenytoin was made at the University of Kansas in our laboratory by a then undergraduate student, Robert Meyers, who later went on to get his PhD under my supervision. While American Critical Care was able to pursue a number of initial animal and I believe some early human studies, the license was transferred to Dupont, and it is my understanding that the rights to the patent and to fosphenytoin did, after 10+ frustrating years, end up at what was then Warner Lambert Parke Davis (WLPD), who gained FDA approval of injectable fosphenytoin in about 1995 for both IV and IM use. It was sold under the brand name of Cerebyx® but is now generically available.

Numerous animal and human preclinical and clinal studies of fosphenytoin have been published from multiple research groups. Some of the most significant early findings are shown as a composite in Fig. 8. It includes selected studies in animals and humans comparing phenytoin levels and safety findings after fosphenytoin and sodium phenytoin administration.[38,40,43–52] Fig. 8a shows a cross-over IV study in a single rat, which was needed because of significant pharmacokinetic differences between rats. Across six rats, absolute phenytoin bioavailability ranged from 80% to 120%. Fig. 8b shows phenytoin levels in rats after IM injection and demonstrates the superior blood levels resulting from fosphenytoin. Fig. 8c shows phenytoin levels across 12 subjects receiving infusions over 30 min. While the AUC values were identical, one can observe that the bioconversion rate of fosphenytoin to phenytoin contributes to time differences for the C_{max}. Fig. 8d compares phenytoin plasma levels from a 10 min IV infusion of sodium phenytoin to an equimolar dose of fosphenytoin given IM. Absolute bioavailability from IM fosphenytoin is close to 100%. Fig. 8e is actually three figures showing that IV fosphenytoin has superior safety to IV sodium phenytoin in humans as measured by pain on injection, phlebitis at the injection site, and vein cording.

Although the initial market acceptance of fosphenytoin was slow and limited due to the cost differential between Cerebyx® and generic sodium phenytoin injectable, generic fosphenytoin is now the leading injectable form of phenytoin used to treat grand mal seizures as well as other conditions.

What are the Advantages of Fosphenytoin Over Sodium Phenytoin?

The advantages of fosphenytoin over sodium phenytoin can be described in four words: "greater safety, greater flexibility." Being able to safely and rapidly infuse or inject fosphenytoin is a clear advantage, but not having to worry about lung emboli, exposure to propylene glycol and a pH 12 solution is significant. The convenience of being able to give the product by the IM route when IV administration is inconvenient or impossible is also significant. The

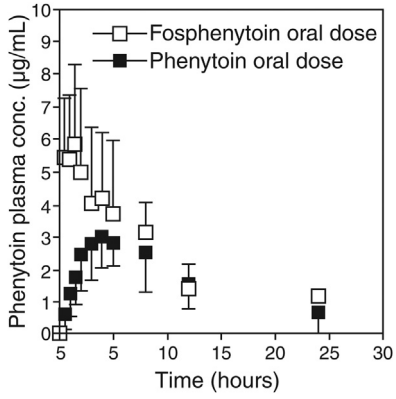

Fig. 11. Phenytoin plasma concentration versus time after oral administration of equimolar amounts of phenytoin and fosphenytoin to six rats. From the work of Burstein et al.[70] Reproduced with permission.

IM route is also used as a temporary replacement for oral administration when a patient is not able to take their oral sodium phenytoin.

What are Some of the Limitations of Fosphenytoin?

Like all drugs, fosphenytoin has some limitations. Since it releases phenytoin, it will exhibit the same systemic side effects as phenytoin.[30] In addition, one unique side effect with fosphenytoin is transitory pruritis/parethesia, a tingling sensation following IV administration. The effect tracks the time profile of the fosphenytoin and not the phenytoin.[46] This effect is not unique to fosphenytoin but has been observed after parenteral administration of some other phosphate prodrugs.[53] While disconcerting, this effect is not limiting.

The commercial fosphenytoin is sold as a ready-to-use sterile solution that requires storage under refrigeration to effect an adequate two year shelf life.[54–56]

A Novel Fosphenytoin Reformulation Strategy?

As indicated above, while the chemical stability of fosphenytoin is maximal around physiological pH, the primary degradation product in this pH range is sparingly soluble phenytoin.[54,55] Thus, fosphenytoin was formulated at a higher pH of around 8.3–9.[56] While overall stability was compromised at this pH, precipitate formation was avoided because the major hydrolysis pathway in this pH range leads to a is more water soluble degradation product than phenytoin.[54] However, this compromise resulted in the need for refrigerated storage conditions to effect a two year shelf-life. This requirement limits the use of fosphenytoin where refrigeration is inconvenient or impossible, for example, in an ambulance attending an automobile accident for a patient with head trauma with likely seizure implications. Here, rapid treatment of seizures or toning down damaged brain tissue is paramount to the life and long-term recovery of the patient.

Around 1997/8 and documented in an issued patent, we explored a novel concept that sulfobutylether-β-cyclodextrin (Captisol®) could be used to stabilize fosphenytoin at physiological pH without the need for refrigeration.[57,58] This was accomplished not by slowing the degradation of fosphenytoin to phenytoin but by selectively increasing the solubility of the formed phenytoin, thus preventing its precipitation. This effect is illustrated in Fig. 9.

The overwhelming quantity of fosphenytoin in the formulation did not excessively compete with the binding of phenytoin because fosphenytoin has two negative charges, thus inhibiting its competitive displacement of phenytoin with negatively charged Captisol®, which has an average of seven negatively charged sulfonate groups.[57,58] Therefore, phenytoin can be effectively solubilized by Captisol®. Table 2 shows the phenytoin-solubilizing effect of Captisol® in the presence of high fosphenytoin concentration.

While this concept has yet to be commercially approved, it is my understanding that it is being pursued and may still result in the introduction of a fosphenytoin product that is stable at room temperature.

Oral Administration: An Opportunity Lost

The oral bioavailability of phenytoin from sodium phenytoin can be erratic.[59–64] This is especially the case in children and patients with short gastrointestinal transit times such as in the case of institutional diarrhea outbreaks.[65] Erratic bioavailability is further exacerbated by the narrow therapeutic window and non-linear pharmacokinetics of phenytoin.

Since the development of fosphenytoin, phosphate ester prodrugs of sparingly soluble and poorly orally bioavailable drugs have been studied and found to provide less erratic and superior plasma levels after oral administration.[66–70] An excellent example is the recently approved drug fostamatinib sold as Travalisse®.[71–73] The parent active drug showed almost no absorption on oral dosing from solid dosage forms due to extremely limited solubility, whereas its phosphoryloxymethyl prodrug gave excellent oral bioavailability.[74]

Why are phosphate prodrugs given orally so effective for oral administration of solubility-limited drugs? The mechanism for this enhancement has been well studied and was elucidated by various researchers at the University of Michigan.[75–77] The cells on the surface of the villi in the small intestines of all animal species, including humans, are rich in the enzyme alkaline phosphatase. As envisaged and proven by researchers at Michigan, water-soluble phosphate prodrugs, on approaching the villi surface, are dephosphorylated by cell-surface-embedded phosphatases that release the parent drug, which is absorbed if it is readily permeable. This coupling of metabolism and transport is used by animal species to absorb polar nutrients.[75] A model depicting this concept can be seen in Fig. 10.

The idea of developing fosphenytoin for oral use was not fully formed or appreciated by us at the time of its discovery, although excellent oral bioavailability of phenytoin was noted in rats and dogs in one of our early animal studies.[38,40] Subsequently, Burstein et al.[70] showed in a nice study that fosphenytoin results in superior phenytoin levels in rats as illustrated in Fig. 11, and similar findings are seen in human volunteers.[78]

Unfortunately, there is little commercial incentive to evaluate fosphenytoin as an improved oral form of phenytoin. I consider this an opportunity lost.

Personal Testaments

Most of us go through life with honorable goals in mind but also focused on the next hurdle, passing exams, getting a degree/s, first job etc. In my case, it was getting my doctorate, teaching and research, striving for tenure, the next grant and occasionally the accolades that come with success. And then there are moments that make one stop and reassess.

I used to have dinner once a month with some male friends, one of whom was a local surgeon. During predinner drinks, he walked into the room and slapped me on the shoulder and said, “we saved a life today, Val!” I reminded him that he did that every day, and he related his experience that he had a patient that afternoon who went into grand mal seizures on the operating table. His attempts to control the seizures with his treatment of choice at the time were not working, and he was about to lose her. He tried fosphenytoin, and it immediately turned off the seizures. “She's going to be fine.” “WE saved a life today.” One does not forget such moments.

One of my former students had a young daughter that fell from a retaining wall at a park and suffered a head fracture. When she was admitted to the local hospital, the ER doctor told her parents he wanted to give her an anticonvulsant to help tone down the brain and prevent the chance of seizure onset. That drug was fosphenytoin. The daughter recovered nicely. The student had helped in our work on fosphenytoin 25 years prior to his daughter's accident! His email to me reminded me that it is not the papers, grants and accolades that are important, but it is the impact one makes in the lives of others.

These two events and a number of others made me reassess what was important in my life's work.

Conclusion

The route to my initial interest in prodrugs and to the discovery of fosphenytoin took many turns, but it started with the mentoring and support of Professor Takeru Higuchi and the hard work of a number of my former students and collaborators. It truly takes a village to discover a drug, but it takes an army to carry the idea to the marketplace and impact the lives of many. My hope is that this paper inspires others to add to the body of science needed to discover new agents to treat unmet medical needs.

Acknowledgements

I would like to thank all my professors for providing me with the tools, guidance, proper foundation, and collegial atmosphere that helped lead me to the type of discoveries like fosphenytoin. In particular, I would like to acknowledge the mentoring of the late Professor Takeru Higuchi. My life as a scientist has been enriched by what I learned from each of my graduate students, post-doctoral researchers, visiting scientists from all over the world, technicians and colleagues. Relative to fosphenytoin and its development, I would like to particularly thank Sailesh Varia, Steve Schuller, Rob Myers and Ken Sloan. It has been a journey that I have enjoyed immensely.

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