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Regulatory perspectives of Type II prodrug development and time-dependent toxicity management: Nonclinical Pharm/Tox analysis and the role of comparative toxicology

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Abstract

Many therapeutic agents are prepared in prodrug forms, which are classified into Type I, II and subtypes A, B based on their sites of conversion. Recently, an increasing number of INDs have appeared as Type II prodrugs that often contain dual tracks of toxicity profile exploration, one on the prodrug and another on the active drug. A comparative toxicology analysis is introduced here to assist reviewers to evaluate the dual toxicity profiles effectively. The analysis helps determine which toxicity is contributed by the prodrug itself, its intermediates, or the active drug itself. As prodrug INDs, or any other new molecular entity (NME) INDs progress into advanced phases of toxicology development, analysis of time-dependent component of toxicity expression, regarding the emergence of new target organs over time, becomes more significant. A strategy is developed to address Pharm/Tox issues such as what duration is required for a toxicity to emerge at the exposure level achieved or dose studied, how many animals in the group are affected, whether the toxicity is a cross-species phenomenon, and whether it is reversible, etc. In conclusion, dual-track comparative toxicology can be useful in the understanding of Type II prodrug's mechanism of toxicity, and that time-dependent toxicology analysis offers means to detecting new toxicity emergence over time. Both approaches could significantly facilitate secondary and tertiary review processes during IND development of a prodrug or NME.

1. Introduction

Many therapeutic agents prior to presenting at the pharmacological site of action in the body are administered often as prodrug. This can happen serendipitously during drug development or can be a result of rational drug design. Examples of the former case, prodrugs that were not originally intended as such during drug development, include aspirin, psilocybin, irinotecan, codeine, heroin, and various antiviral nucleosides. Examples of the latter cases, which were part of a strategically targeted drug design, include sulfasalazine, oseltamivir, various NSAIDs (ketoprofen, diclofenac), statins (lovastatin, simastatin), ACE inhibitors (captopril, lisinopril) and penicillin-related agents (bacampicillin, sarmoxicillin). The need to design a prodrug is often related to the issues related to bioavailability, such as poor aqueous solubility (corticosteroids), poor absorption/permeability (ampi-

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Table 1
Classification of prodrugs

Prodrug classification	Site of conversion	Subtype	Tissue location of conversion	Examples
Type I	Intracellular	Type IA	Therapeutic target tissues/cells	Zidovudine, 5-flurouracil
		Type IB	Metabolic tissues (liver, lung, etc.)	Captopril, cyclophosphamide
Type II	Extracellular	Type IIA	GI fluid	Sulfasalazine, loperamide oxide
		Type IIB	Systemic circulation	Fosphenytoin, bambuterol

A prodrug can belong to both a Type IA and IB category when the site of the therapeutic target and conversion are the same (e.g., HMG Co-A reductase inhibitors).

cillin) and high first pass extraction (propanolol); or that the active drug is nonspecific (anticancer agents), is incompletely absorbed (epinephrine), exhibits unfavorable organoleptic properties (chloramphenicol), has a short half-life (dopamine) or other adverse effects or toxicities (AHFS, 2007; Brunton et al., 2005; Goldstein et al., 1974).

2. Type I and Type II prodrugs

Prodrugs can be classified into two types based on their sites of conversion into the final active drug form: Type I, those that are converted intracellularly (e.g., anti-viral nucleoside analogs, lipid-lowering statins, antibody-directed/gene-directed enzyme prodrugs [ADEP/GDEP] for chemotherapy); and Type II, those that are converted extracellularly, especially in digestive fluids or the systemic circulation (e.g., etoposide phosphate, valganciclovir, fosamprenavir). Both types can be further categorized into subtype A or B, based on additional criteria. Those for the Type IA and IB are whether or not the cellular converting location is the site of therapeutic action. For the Type IIA and IIB, they are categorized depending on whether the conversion occurs in the gastrointestinal (GI) fluids or systemic circulation (see Table 1).

Although Type I prodrugs can serve as an avenue to rational drug design, Type II prodrug design has been shown to be a convenient and efficient means to circumvent bioavailability issues. In many therapeutic classes, overcoming poor permeability and vulnerability to gastric acidity has become a major goal of pharmaceutical development. Recently, an increasing number of Investigational New Drug Applications (INDs) have appeared in the U.S. markets as Type II prodrugs. Common characteristics of Type II prodrug submissions and regulatory insights derived from evaluation of these INDs are discussed here. In addition, issues related to time-dependent aspect of toxicity exploration during prodrug and other NME drug development are also discussed.

3. Preclinical pharmacokinetic considerations of Type II prodrug INDS

Because the eventual therapeutic effect of a Type II prodrug is expressed through the final active drug, certain pharmacokinetic information becomes particularly important to the review of the IND. This information includes at least the following: (1) the site where the prodrug is converted into active drug (e.g., GI fluid, systemic circulation, etc.); (2) the enzymes involved in the conversion and kinetics of the catalytic processes (e.g., esterase, phosphatase, etc.; the kinetics of the conversion at each site and whether multiple sites are involved); and (3) the extent of the transition and the duration of prodrug molecule or its intermediates, if any, appearing in the systemic circulation. This should be expressed as the detectable plasma concentrations (e.g., what are the percentage and the half-life of the prodrug and each intermediate in each converting site). In some cases, drug concentration measurements may be made in hepatic portal and post-hepatic/systemic vein sites to determine the proportion or extent of conversion. This information is important in the safety assessment of the IND, especially when there is concern on the contribution of the prodrug and intermediates to the overall toxicity profile of the drug product. In regard to perspectives related to analytical assay sensitivity, validation limits for and relative concentrations of the prodrug, active drug and its metabolites, the concerns could be varied dependent upon factors such as the drug's chemical nature, indication, metabolic and toxicity profile, and fulfillment of exercising good faith efforts in solving feasibility issues.

4. Comparative toxicology of prodrug and active drug

The contributing role of prodrug, or intermediates that are transiently present in the body, to the overall toxicity profile of the drug product (prodrug) can be analyzed through evaluating the comparative toxicology of prodrug and active drug. The comparative toxicology

reflects a unique and identifiable feature submitted with many Type II prodrug IND packages, in which the preclinical safety information contains toxicity profiles on the prodrug as well as on the active drug. This dual-track toxicology sometimes came about when the active drug itself was developed in an early study and found inadequate. Later, a prodrug was developed due to reasons delineated above (e.g. bioavailability). In CDER, FDA, guidance documents, such as those available at the FDA website (http://www.fda.gov/cder/guidance/index.htm#Pharmacology/Toxicology) or in publications (e.g., Wu et al., 2004), for prodrugs have not been formulated. Dual-track comparative toxicology is not a nonclinical pharmacology/toxicology requirement for a prodrug IND. Rather; it is often resulted from the sponsor's own initiative to fully understand the drug product's toxicity profile. The importance of comparative toxicology can be highlighted by the experience with terfenadine/fexofenadine in which elevated levels of "prodrug" terfenadine resulting from inhibition of its conversion to the "active drug" fexofenadine (e.g., by ketoconazole or erythromycin) could produce life-threatening QT prolongation, Torsade de pointes arrhythmias or sudden cardiac death (the "prodrug" terfenadine has been withdrawn and replaced by the "active drug" fexofenadine in the U.S. market)(http://www.fda.gov/bbs/topics/news/new00286.html).

In most Type II prodrug INDs, the converting enzymes involved are both ubiquitous (e.g., esterases or phosphatases) and fast-acting. Thus, the unconverted prodrug and intermediates represent only a minor portion of the drug products circulating in the body. Because of this, the active drug's toxicity profile is often claimed by the sponsor to reflect or to represent the toxicity profile produced when prodrug alone is administered. A comparative toxicology table is designed here on "prodrug administered alone" and "active drug administered alone" to provide a useful reference for reviewers to evaluate the dual toxicity profiles effectively and to derive regulatory insights. An example is provided here for discussion (Table 2). The table has a "prodrug alone" and an "active drug alone" column indicating animals that were treated with active drug only or with prodrug only. The table rows are aligned with categories of specific target organs of toxicity and toxicity profile descriptions. Under each specific toxicity category, threshold (dose that elicited the toxicity) and NOEL as obtained from the respective toxicology studies are provided. The systemic drug exposures at that NOEL is also provided. By comparing "prodrug alone" and "active drug alone" columns on target organs or profiles of toxicity, any difference

Table 2
Comparative toxicology between orally administered prodrug and active drug

Target organs	Profile of toxicity	Prodrug alone (Threshold/NOEL/AUC of NOEL)*	Active drug alone (Threshold/NOEL/AUC of NOEL)
GI	Emesis, diarrhea and mucoid feces	4-week dog study, 1000/300/547	4-week dog study, 60/-/-
		Single-dose dog study, 1000/300/600	2-week dog study, (600/200/451)
		2-week dog study, 1000/300/575	
	Soft feces	4-week rat study, 200/-/-	4-week rat study, 150/-/-
Erythropoietic Tissues and Bone Marrow	Reduced erythrocyte counts and hemoglobin concentration	4-week rat study, 2000/500/400	4-week rat study, -/1500/161
	Bone marrow suppression	2-week dog study, 300/100/228	2-week dog study, 600/200/451
Liver	Increased organ weight	4-week rat study, 2000/500/400	4-week rat study, -/1500/161

* Threshold/NOEL/AUC OF NOEL represents the threshold dose (mg/kg), NOEL (mg/kg) and AUC (measured active drug exposure expressed in μg h/ml for this case) listed in a sequence of 3 numbers. When the sequence contains only 2 numbers (-/y/z), NOEL would be the maximum dose tested (i.e., toxicity did not occur in all doses tested); if there was no NOEL (i.e., toxicity occurred at all doses tested), the sequence is represented by x/-/-.

Table 3
Time-dependent emergence of toxicity

WEEK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Liver Enzyme Induction (Hypertrophy, ↑organ weight)																										
Rat	№*	▲*	→	▲	→**	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		100/13-27*		<100/1.7-3.6																						<75/8-22
Monkey	▲	№	→	▲	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
	200/36-40	1000/20-23		100/11-13																						10/1.5
*: NOEL [mg/kg]/AUC [estimates of mean values for both sexes, ug·h/ml); №: Absence of toxicity; ▲: Presence of Toxicity.																										
Liver Injury (Hepatocellular vacuolation, ↑liver enzymes/bilirubin)																										
Monkey	▲	▲	→	▲	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
	50/18-22	10/3-6		<20/3																						10/1.5
Liver Injury (Hepatocellular inclusions/deposits/granuloma, bile duct hyperplasia)																										
Rat	№	№	→	№	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		2000/95-105		1000/8-15																						75/8-22
Thyroid Toxicity (↑Organ weight, ↓T4, ↑TSH)																										
Rat	№	▲	→	▲	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		100/13-27		<100/1.7-3.6																						75/8-22
Kidney Toxicity (↑Organ weight, chronic progressive nephropathy)																										
Rat	№	№	→	▲	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		2000/95-105		100/1.7-3.6																						75/8-22
GI Toxicity (Granuloma inflammation with gold/brown crystals in duodenum, jejunum, mesenteric lymph node)																										
Rat	№	№	→	№	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		2000/95-105		1000/8-15																						75/8-22
Hematotoxicity (↓RBC counts/Hct/MCH)																										
Rat	№	№	→	№	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		100/13-27		1000/8-15																						300/14-40
Coagulation Abnormalities (↑APTT/PT)																										
Rat	№	▲	→	▲	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
		100/13-27		≈100/1.7-3.6																						75/8-22
Monkey	№	№	→	№	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	▲
	1000/30-101	50/14-16		100/11-13																						10/1.5

*Symbol "▲" represents emergence of toxicity, 'No' represents absence of toxicity. Sequence of two numbers represent NOEL and AUC (please see legends of Table 2 for additional details).
*Symbol "→" represents progression of time among the only four lengths of toxicity studies provided here (i.e., 1-week, 2-week, 4-week and 26-week).

becomes apparent and evident. However, factors that lead to these differences could be summarized by speculations that: (1) additional toxicity may be contributed by the prodrug molecule itself or its intermediates (this likelihood could be supported by measurable blood levels of these compounds in the systemic circulation); (2) additional toxicity may be contributed by a significant difference in final active drug exposure levels in the "prodrug alone group" or the "active drug alone group" animals, whereas both prodrug and intermediates play little role when conversion was proven fast and there was no measurable prodrug or intermediates in the blood. For example, Table 2 shows significant gains in liver organ weight and decreases in erythrocyte counts and hemoglobin concentrations that are observed in the prodrug rather than the active drug study, which may be due to lower maximum drug exposures achievable in the active drug alone group (400 *versus* 161 μg h/ml) that was not sufficiently high to induce the effect.

In some cases, intravenous administration of active drug in the active drug alone group may become helpful (e.g., due to poor oral bioavailability) in providing comparable exposures between prodrug alone group and active drug alone group. Unless additional or new target organs of toxicity emerge in longer term toxicity studies, or concerns are raised upon levels of prodrug or its intermediates, further dual-track toxicology should not be necessary in the later phases or longer terms of preclinical studies.

5. Time-dependent component of the toxicity progression and development

As presented in the previous section, supplemental dual-track toxicology (studies conducted using prodrug or active drug alone) is often available in the initial stage of prodrug INDs. As development progresses and longer-term toxicity studies are performed, dual-track toxicology may no longer be available as long-term toxicity studies focus only on the prodrug alone. It is during this more advanced phase of toxicology development that the issue of time-dependent toxicity expression becomes more meaningful. Generally speaking, detecting the emergence of new toxicity and exploring the full toxicity profile of prodrug or other NMEs rely on in-depth evaluation of not only the dose-dependent but also the time-dependent components of the toxicities. The dose-dependent component includes the well-known dose escalation schedule that allows achievement of high drug exposures. The time-dependent component refers to detecting toxicity emergence by performing "comparative toxicology" among studies of different duration. To analyze this time-dependent behavior, a scheme is designed to capture key information by using a tracking spreadsheet (see case example provided in Table 3) to relate various timepoints at which a particular toxicity emerges. The table shows that the specific toxicity is arranged under the row category, while the time dimension is proportionally represented by the succession of columns on the *y*-coordinate. Using this format, a specific toxicity that occurs during a study of one duration but not in other shorter study durations can be tracked/highlighted by a milestone/signal at that time point. Auxiliary information such as threshold dose/NOEL/AUC, number or percentage of subjects affected by this toxicity could be provided at this time point (e.g., underneath each toxicity row) to facilitate an overview. For example, from Table 3, hepatic enzyme induction required one week to appear in rats and 2 weeks in monkeys. Hepatocellular injury occurred within one week in monkeys, whereas it did not happen in the first month but eventually emerged from the 6-month study in rats. For the renal, GI, and hematotoxicity it required 2, 4, and 26 weeks of drug treatment for the effects to emerge and be detected in rats. Progression of thyroid toxicity proceeded in a similar fashion, although its relevance to human risk has been questioned (Wu and Farrelly, 2006a). Coagulation abnormalities required 2 weeks in rats but 26 weeks to become detectible in monkeys. Both hepatic enzyme induction and coagulation abnormalities are cross-species phenomena.

Thus, an overall toxicity profile, including that explored in different species, can be organized by combining all equivalent data, as presented in Table 3, in a single spreadsheet. Questions such as what is the treatment duration that is required for a toxicity to emerge at the exposure level achieved or dose studied, how many animals in the group are affected, whether the toxicity is a cross-species phenomenon and whether it is reversible, can be answered from this presentation. It is concluded that by using this approach, the tracking mechanism could provide a concise presentation of a drug's toxicity profile and would significantly facilitate the Agency's secondary and tertiary review processes during drug development.

6. Conclusions

Employing comparative toxicology has proven useful in the determination of target organ of toxicity and toxicity profile produced by Type II prodrug alone or active drug itself. Further, the time-dependent toxicity analysis offers an additional insight into the understanding of the

progression of toxicity expression and provides a different dimension in toxicity management on prodrug and other NME INDs.

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