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Toxicokinetics and Toxicodynamics

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toxicodynamics of a given toxic xenobiotic are dependent on the mechanism of action of that toxicant and the relationship between toxicant concentration and the observed effects of the toxicant on biological processes in the animal (i.e., the dose-response relationship). The disposition and/or toxicokinetics of a particular xenobiotic also play a role in determining the organs or tissues affected by a toxicant, and the clinical presentation and time course of a toxicosis resulting from excess exposure to that compound.[1]

DEFINITIONS

The basic concepts regarding the toxicokinetics and toxicodynamics of xenobiotics are clinically relevant to veterinary toxicology and need to be understood by veterinary practitioners, professional students, and other personnel who will be participating in the diagnosis and treatment of small animal intoxications. In discussing the aspects of toxicokinetics and toxicodynamics most pertinent to small animal toxicoses, it is first necessary to define several terms. "Xenobiotic" is a general term referring to any chemical foreign to an organism or, in other words, any compound not occurring within the normal metabolic pathways of a biological system.[1,2] Depending on the compound and the level of exposure, interactions between xenobiotics and animals can be benign, therapeutic, or toxic in nature. The pharmacokinetics and pharmacodynamics of a therapeutic xenobiotic influence the time course and efficacy of that compound in a pharmacological setting. Likewise, the toxicokinetics and toxicodynamics of a toxic xenobiotic determine the "when," "how long," "what," and "why" for the adverse effects of that toxicant.[2]

The "disposition" of a xenobiotic is what the animal's body does to that compound following exposure. The disposition or fate of a xenobiotic within the body consists of the chemical's absorption, distribution, metabolism (biotransformation), and excretion characteristics, which are collectively abbreviated as ADME.[2,3] "Toxicokinetics" refers to the quantitation and determination of the time course of the disposition or ADME for a given toxic xenobiotic.[3] There are a variety of specialized toxicokinetic terms, including bioavailability, volume of distribution, clearance, half-life, one-compartment model and first- and zero-order kinetics, which will be discussed later in this chapter under the separate components of ADME.

The term "toxicodynamics" describes what a toxicant does physiologically, biochemically, and molecularly to an animal's body following exposure.

TOXICOKINETICS/DISPOSITION

Xenobiotic absorption

With the exception of caustic and corrosive toxicants that cause adverse effects at the site of exposure, a toxic xenobiotic is generally first "absorbed" or taken up into the body.[3] Absorption involves crossing cellular membranes, which are typically composed of phospholipid bilayers containing various sized pores and embedded proteins.[3] The route of exposure and physiochemical properties of a toxicant, such as its resemblance to endogenous compounds, its molecular size and relative lipid and water solubilities, the magnitude of a molecule's association constant, and whether a compound can be classified as a weak acid or as a weak base, determine the manner and quantities in which a xenobiotic is absorbed across cell membranes.

Routes of xenobiotic exposure and xenobiotic bioavailability

The most common routes of exposure for xenobiotics in small animal toxicology are oral (gastrointestinal), dermal (percutaneous), and inhalation (pulmonary). In rare instances of iatrogenic intoxications, xenobiotics can be injected subcutaneously, intramuscularly, intraperitoneally, or even intravenously.[3] There are unique aspects to the absorption of xenobiotics associated with each route of exposure, especially with regard to the bioavailability of potential toxicants.

"Bioavailability" (often represented by "F" in toxicokinetic equations) represents the fraction of the total dose of a toxic xenobiotic that is actually absorbed by an animal.[2] In intravenous exposures, the bioavailability of a toxic xenobiotic is 100% since the entire dose of the toxicant reaches the peripheral circulation. The absorption of gases and vapors in the

respiratory tract is largely dependent on the ratio (blood-to-gas partition coefficient) between the equilibrium concentrations of the toxicant dissolved in the blood and the gaseous phase of the toxicant in the alveolar spaces.[2,3] The size of aerosolized particles will determine to a large degree whether a xenobiotic is deposited in the nasopharyngeal region (particles > 5 μm) or within the alveoli of the lungs (<1 μm).[2] The stratum corneum and its associated keratinized structures often impede the percutaneous absorption of xenobiotics, and there are variations in the absorptive ability of skin in different anatomical locations.[4] Dermal absorption is frequently dependent on the vehicle in which a toxicant is dissolved and is generally greater for lipid soluble compounds as compared with chemicals that are highly soluble in water.[2,4] The bioavailability of toxic xenobiotics that are ingested can be negatively impacted by acidic degradation in the stomach and/or enzymatic breakdown in the small intestine.[2] Decreased gastrointestinal transit time can diminish xenobiotic bioavailability by limiting the access of toxicants to those regions of the digestive tract where rates of absorption are greatest. Some potential toxicants, especially certain heavy metals (e.g., lead and cadmium), resemble essential minerals such as calcium and zinc, respectively. The gastrointestinal absorption of these toxic nonessential metals involves interactions with dietary levels of the corresponding essential metals and regulated mechanisms of gastrointestinal uptake designed for these required minerals.

Hepatic biotransformation of xenobiotics, which will be discussed in greater detail later in this chapter, can also influence the apparent bioavailability of ingested toxicants. Following oral exposure, xenobiotics absorbed from the gastrointestinal tract are transported to the liver via the hepatic portal circulation. For some xenobiotics, rapid hepatic degradation (and in some instances prior biotransformation in gastrointestinal cells) prevents access of the compound to the systemic circulation, resulting in an apparently decreased bioavailability from what is termed the "first-pass effect" or "presystemic elimination."[3,4] In contrast, the bioavailability of some chemicals is enhanced by a cycle of biliary excretion and subsequent reuptake from the intestines referred to as "enterohepatic recirculation."[4]

Mechanisms of xenobiotic absorption

The passage of xenobiotics through cellular membranes can be either energy-independent ("passive" transport) or can require the expenditure of energy through "specialized" or "active" transport systems. Passive transport of xenobiotics can be accomplished through simple diffusion or filtration. Specialized, energy-dependent, cellular transport systems include the process specifically referred to as "active transport," along with facilitated transport and pinocytosis.

Passive transport of xenobiotics

Simple diffusion and filtration are nonsaturable processes, which do not require the expenditure of energy to transport xenobiotics across cellular membranes.[2,3] Both of these mechanisms of passive transport are dependent on the concentration gradient for a given xenobiotic, with the rate of transport being proportional to the difference in that chemical's concentration between the two sides of a particular membrane (Fick's law). Simple diffusion is the most common mechanism by which xenobiotics cross cellular membranes. Uncharged (nonionized), lipid-soluble molecules, especially those which are small, are more readily diffusible across phospholipid bilayers of biological membranes than charged (ionized) molecules, which are generally less lipid-soluble.[3] The Henderson-Hasselbalch equation can be used to predict whether a particular xenobiotic will be in the nonionized or ionized state in a particular biological matrix. In this equation, the difference between the association constant (pK_a, which is equivalent to the pH at which equal amounts of a xenobiotic are in the nonionized and ionized states, and the pH of the biological matrix in which the xenobiotic will exist (i.e., pK_a – pH) is equal to the common log of the quotient of nonionized xenobiotic divided by ionized xenobiotic for weak acids and the log of the reciprocal quotient (ionized xenobiotic divided by nonionized xenobiotic) for weak bases.[3,4] Filtration involves the passage of xenobiotics through patencies or pores within cellular membranes and is determined, in large part, by the size of the xenobiotic molecule and pore-size, which varies in different organs and tissues.[2]

Specialized transport of xenobiotics

Active transport is an energy-dependent, saturable process by which xenobiotics are transported across biological membranes against electrochemical or concentration gradients.[2,4] Specific examples of active transport systems include the multidrug-resistant proteins (P-glycoproteins) and members of the organic cation transporter family.[3] Facilitated or carrier-mediated transport can require the expenditure of energy, but, in contrast

to active transport. xenobiotic transport by this mechanism is not ag[ainst]
a concentration gradient.[2,3] Pinocytotic transport involves cellular eng[ulf]-
ment of small amounts of xenobiotic and the transfer of this amoun[t of]
chemical through the cellular membrane.[3]

Xenobiotic distribution

"Distribution" refers to the translocation of a xenobiotic from the site[s of]
absorption to various body organs and tissues and involves both transp[ort]
of the chemical within the circulation and cellular uptake of the xen[o]-
biotic.[1-3] The rate of xenobiotic transfer into a particular organ or tiss[ue]
is determined by the physiochemical properties of the specific xenobio[tic]
(e.g., lipid solubility and molecular weight), the blood flow to the organ[s or]
tissues in question, and the rate of diffusion of the xenobiotic across t[he]
endothelial walls of the capillary bed into cells within a particular org[an]
or tissue.[2-4] The "volume of distribution" (V_d) for a given xenobiotic re[p]-
resents the quotient of the total amount of that chemical in the bo[dy]
divided by the concentration of the xenobiotic within the blood and[is]
used to describe the extent to which a xenobiotic is distributed within t[he]
body.[2,4] The V_d is a clinically relevant indicator as to whether a chemic[al]
is primarily contained within the plasma compartment (relatively low V[d])
or whether a compound is widely distributed throughout the body with[in]
the interstitial and/or intracellular compartments of various organs a[nd]
tissues (relatively high V_d).[2,3]

Xenobiotic storage depots

Xenobiotics can be stored within a variety of different body orga[ns]
and tissues. Depending on the anatomical and physiological relationshi[p]
between the storage depot and the target organ(s) and/or tissue(s) f[or]
a specific toxicant, storage of toxic xenobiotics can function as either[a]
protective mechanism or as a means by which the toxic effects of a xen[o]-
biotic are potentiated. An understanding of the storage sites of toxic xen[o]-
biotics can provide additional insight about circumstances that would [be]
expected to exacerbate a particular toxicosis along with indicating whi[ch]
organs or tissues would be expected to have the highest concentrations f[or]
diagnostic sampling. Plasma proteins represent a storage site for ma[ny]
xenobiotics (e.g., salicylates, barbiturates, cardiac glycosides) and importa[nt]
physiological constituents, including steroid hormones. vitamins, a[nd]
various essential minerals.[3] Displacement of toxic xenobiotics fr[om]

[plas]ma proteins can greatly increase the amount of unbound toxicant dis-
[trib]uted to target organs or tissue. A wide variety of xenobiotics accu-
[mu]late in the liver and kidneys. making these organs ideal sites for
[post]mortem sample collection in cases of suspected toxicoses. Some toxic
[met]als, such as cadmium. accumulate in the liver and kidneys because of
[the] high endogenous concentrations and induction of metallothionein in
[the]se organs. Fat and bone are storage depots for a variety of different
[xen]obiotics, and rapid depletion of body fat stores (weight loss) or
[incr]eased remodeling of bone during growth or pregnancy have the
[pot]ential to increase the exposure of target organs or tissue to previously
[stor]ed toxicants.[3,4]

Potential tissue barriers to xenobiotic distribution

[Th]e blood-brain barrier is frequently mentioned in the current literature
[with] regard to its ability to limit exposure of the central nervous system
(CNS) to toxic xenobiotics. Other potential barriers to chemical uptake
[als]o occur in the eyes. testes. prostate. joints. and placenta. In these
[ins]tances only small. nonionized. lipid-soluble molecules are able to cross
[the] membranes and gain access to potential target tissues.[1]
The "blood-brain barrier" to xenobiotic uptake consists of the rela-
[tiv]ely nonporous CNS capillary endothelium. which contains multidrug-
[res]istant protein and is surrounded for the most part by glial cells.[1] The
[ext]remely low protein content of the interstitial fluid within the CNS also
[con]tributes to the apparent inability of many protein-bound. toxic xeno-
[bioti]cs to reach clinically relevant concentrations in the brain.[3] Since the
[bloo]d-brain barrier is not fully formed at birth and is less well-developed
[in s]ome breeds of dogs (e.g.. collies and collie crosses). immature animals
[and] collie-related breeds are more susceptible to the adverse effects of
[com]pounds normally "blocked" by the blood-brain barrier.[3,5]

Xenobiotic metabolism/biotransformation

["Me]tabolism" can be used to refer to the fate or disposition of a xenobi-
[otic] or the sum total of the chemical transformations of normal body con-
[stitu]ents, which occur in living organisms.[1,6] Biotransformation, on the
[oth]er hand, is a general term referring to the metabolic conversion of
[both] endogenous and xenobiotic chemicals into more water-soluble
[form]s.[6] For the purposes of this chapter, xenobiotic "metabolism" and

"biotransformation" are synonymous and refer to the generally two-phase process by which chemicals are converted to more water-soluble forms for excretion from the body.[1,2] In xenobiotic metabolism/biotransformation, the lipophilic (lipid-soluble) properties of xenobiotics that favor absorption are biotransformed into physiochemical characteristics (hydrophilicity or water solubility) that predispose compounds to excretion in the urine or feces.[6] Although multiple organs within the body have biotransformation capabilities, most xenobiotics are biotransformed in the liver.[6]

Phase I and phase II xenobiotic biotransformation

Xenobiotics are usually biotransformed in two phases (I and II), which involve enzymes having broad substrate specificity.[2,6] Phase I reactions generally involve oxidation, hydrolysis, or reduction, and convert apolar lipophilic xenobiotics into metabolites, which have greater polarity and hydrophilicity.[2] In these instances, hydroxyl, amino, carboxyl, or thiol moieties are usually either exposed or added to increase water solubility. Oxidation reactions, especially those catalyzed by cytochrome P450 enzymes, are the phase I biotransformations most commonly involved in xenobiotic metabolism, and many xenobiotics are able to induce cytochrome P450 activity.[6] During phase II biotransformation, the xenobiotic or its metabolites are conjugated with a functional group (e.g., glucuronide, sulfate, amino acids, glutathione, or acyl or methyl groups), resulting in a compound with dramatically increased water solubility.[2] Not all mammalian species have equal phase II biotransformation capabilities, and the inability of domestic cats to glucuronidate xenobiotics is especially clinically relevant to veterinary toxicologists.[2,5]

Most xenobiotic biotransformations result in less toxic metabolites. However, there are xenobiotics (e.g., acetaminophen and aflatoxin B_1) for which the products of hepatic phase I metabolism are actually more toxic than the parent xenobiotic.[2,5] In these instances of "metabolic activation," "bioactivation," "toxication," or "lethal synthesis," any factors that increase hepatic biotransformation of the parent compound will enhance the amount of toxic metabolite to which the animal is exposed.[5,7]

Xenobiotic excretion

The final step in the disposition of a xenobiotic is excretion, whereby the xenobiotic or its metabolites are removed from the body via a number of

different routes. Renal excretion is the most common means by which xenobiotics and the products of their biotransformation are eliminated from the body, but toxicants can also be excreted in the feces, biliary excretion, or elimination of unabsorbed xenobiotic, saliva, sweat, cerebrospinal fluid, or even the milk, which is clinically relevant in xenobiotic-exposed dams or queens nursing offspring. In instances of exposures to toxic vapors or volatile xenobiotics, exhalation can also be a major route of elimination from the body.[ ] Xenobiotics and their metabolites can be excreted by more than one route of elimination, and the total excretion is generally broken down into renal and nonrenal routes.

Toxicokinetic aspects of xenobiotic elimination

In regard to toxicokinetics, "elimination" of a xenobiotic generally incorporates both the processes of biotransformation and excretion. "Clearance," which is expressed for the whole body and individual organs in terms of the volume of blood that is cleared of the chemical per unit time, is an indicator of the body's ability to eliminate a given toxicant from the body by processes such as metabolism, excretion, and exhalation. The toxicokinetic aspects of xenobiotic elimination are clinically relevant to the management and diagnosis of veterinary toxicoses. These quantitative indices can be used to predict the duration of a toxicosis and the time period necessary for therapeutic intervention. Toxicokinetic aspects of xenobiotic elimination can also be used to determine the time frame and biological samples that are best suited for diagnosing a specific toxicosis.

When developing toxicokinetic models, assumptions are often made with regard to whether a given xenobiotic best fits a "one-compartment" or a "multicompartment" model. A one-compartment model is the simplest toxicokinetic model and assumes that changes in xenobiotic concentrations in the blood or plasma are accurate reflections of what is occurring in the tissues.[2] Assuming that a one-compartment model is appropriate for a particular xenobiotic, elimination of this compound is most likely via first-order kinetics, where the involved processes are most likely nonsaturable and the rate of elimination at any given time point is proportional to the amount of compound that remains in the body at any point in time.[2,4,8] With first order kinetics in a one-compartment model, it is possible to calculate the elimination "half-life" of a xenobiotic using the volume of distribution and the clearance for a given xenobiotic.[8] In this instance, half-life indicates the time required for the blood or plasma concentration of the xenobiotic to be reduced by one half, with

approximately 97% of a xenobiotic being eliminated from the circulation in five half-lives. The term "half-life" can also be used in terms of elimination of xenobiotic from body storage depots rather than from the blood or plasma. It is important to know the context in which this particular term is being used and the compartmental model involved to understand what process in the xenobiotic's disposition is actually being discussed.

There are some xenobiotics for which the processes involved in their elimination are saturable and the rate of elimination is independent of the amount of chemical remaining in the body at a given point of time. Under these circumstances, the pathways of elimination for a given xenobiotic can be described in terms of zero order kinetics. Only a finite amount of xenobiotic can be eliminated per unit time.

TOXICODYNAMICS

Interactions between xenobiotic toxicodynamics and disposition/toxicokinetics

In contrast to toxicokinetics, the toxicodynamics of a particular xenobiotic describe what that compound actually does to adversely affect an animal's health rather than how the animal handles the exogenous chemical. However, a xenobiotic's toxicodynamics and toxicokinetics are not mutually exclusive. What a toxicant does physiologically, biochemically, and molecularly to a living organism following exposure is not only dependent on that xenobiotic's mechanism of action and its dose-response relationship but also on its disposition and/or toxicokinetics within an exposed animal.[1,2]

The first step in the development of a toxicosis is the delivery of the "ultimate toxicant" to its site of action or "target."[7] "Ultimate toxicant" refers to the parent xenobiotic, its metabolite, or even a generated reactive oxygen species that actually causes cellular damage. The term "target" is often used to describe a molecule that interacts with the ultimate toxicant resulting in adversely affected biological processes within an organism. "Target(s)" can also be an inclusive term referring to the cell types, organs or tissues most susceptible to the effects of a toxic xenobiotic.[5,7]

The distribution and biotransformation of a xenobiotic often limit the delivery of the ultimate toxicant to susceptible target cells, organs, or tissues. Distribution of xenobiotics to storage depots that are physically

removed from potential target sites is one means by which the disposition of a toxicant can be protective and can limit the adverse effects of a particular xenobiotic on an animal. Presystemic elimination or the first-pass effect prevents toxic xenobiotics from ever reaching the general circulation and therefore many potential sites of action. Most biotransformations produce metabolites that are more water soluble and as a result more readily eliminated from the body.

In contrast to circumstances where the disposition of a xenobiotic decreases the risk of toxicosis, there are also instances where the distribution and biotransformation of a given toxicant actually increase the likelihood that an ultimate toxicant will be delivered to the site of action. A chemical's toxicity can be enhanced by specialized transport mechanisms and by physiochemical characteristics that facilitate the accumulation of ultimate toxicants within susceptible cells. The toxicity of a xenobiotic can also be facilitated by processes, such as enterohepatic recirculation, that increase its bioavailability.[4,7] Xenobiotic biotransformations that result in lethal synthesis or bioactivation predispose animals to toxicoses and can, in some instances, actually occur within target cells. While some biotransformations result in metabolites that react more efficiently with target enzymes or receptors, it is more common for intoxication to result in chemical species, such as electrophiles, free radicals, nucleophiles, and redox-active compounds that are indiscriminately reactive with endogenous molecules.

General mechanisms of xenobiotic action

The basis for most toxicoses is cellular damage, and this damage is often most dramatic in cells with high rates of metabolism and replication.[3] A toxic xenobiotic's "mode" or "mechanism of action" is the activity of that compound or its metabolites at the molecular or cellular level that results in adverse effects.[1,5] While most of the chapters of this text will review the specific mechanisms of action of toxicants to which small animals are commonly exposed, there are a number of general ways in which toxic xenobiotics adversely affect cellular structure and function.

Although a toxic xenobiotic can adversely affect cells by changing their biological microenvironment through alterations in pH or occupation of a particular receptor site, as mentioned previously, ultimate toxicants generally interact with target molecules or cells. Some xenobiotics mimic the actions of normal nutrients and endogenous hormones or neurotransmitters. Specific receptors can be stimulated or blocked, and enzymes can be inactivated or inhibited. Electrophiles, free radicals, nucleophiles, and

redox-active compounds are often generated through biotransformation, and these chemical species can react indiscriminately with target macromolecules to exert their toxic effects.[5,7] At the cellular level, chemicals can alter cellular maintenance, both internally and externally, adversely affecting membrane integrity and the ability of cells to regulate their volume and/or their energy metabolism.[7] Cellular injury and death often result from the impaired cellular synthesis of ATP, uncoupling oxidative phosphorylation, and the inability of cells to regulate their intracellular calcium concentrations. The cellular production of vital proteins and the regulation of gene expression within cells can also be disrupted by toxicants.[5,7] Ultimately, high enough exposures to toxic xenobiotics cause cellular dysfunction and injury and, sometimes, disrepair, and these adverse effects can be observed clinically as abnormalities in the structure and/or function of different organs and tissues.[7]

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Toxicological Information Resources

<div style="text-align:right">**3**</div>

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Combining the exponential rate at which the body of toxicology information grows with today's improved tools to disseminate this information, one can easily feel overwhelmed. The challenge is to recognize the most appropriate resources that will lead the practitioner to the most relevant and current information.

Toxicological information is available as primary, secondary, and tertiary literature on a variety of media from textbooks to computer databases. The purpose of this chapter is to introduce veterinary practitioners to some of the resources found to be beneficial when approaching a toxicological problem.

PRIMARY LITERATURE

More than 20,000 biomedical journals are published annually. These are the primary literature sources that bring us detailed accounts of research in specialized areas. The level of detail, such as methodology, results, and discussion, exceeds that found in secondary and tertiary resources. This affords readers greater opportunity to determine for themselves the value of the conclusions offered in the study.

There are considerably more toxicological references written for human medical practitioners than for veterinarians. Any thorough search of a toxicological topic would need to query the primary literature in both toxicology and veterinary medicine. Primary literature may be particularly important to the veterinarian practitioner because historically much of